Consumption of animal products and frauds: DNA-based methods for the investigation of authenticity and traceability in dairy and meat-derived products – a review

Consumo de produtos de origem animal e fraudes: uma revisão dos métodos moleculares para a investigação de autenticidade e rastreabilidade em produtos lácteos e cárneos

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Abstract

The increase in the population's acquisition power in emerging countries like Brazil has resulted in increased consumption of meat, milk and their derivatives, and a consequent growing surveillance regarding the responsibility of maintaining the quality of these food products. The total or partial replacement by other than the species declared on the product label in meat, milk or derived products compromises the nature and quality of these products, hurting consumer choice rights, which may be based on medical and nutritional recommendations, the economic value of the product or habits and/or dietary restrictions of each specific culture. Species identification in dairy and meat products is important in food traceability. Although food matrices are complex and variable, biomolecular techniques are gradually being applied for species identification, having proven increasingly reliable, fast, specific and highly sensitive, even in mixed samples. For these reasons, this review intends to show the main molecular methods applied to adulteration detection in dairy and meat derivatives, including an already established method, such as the polymerase chain reaction (PCR), as well as more advanced technologies, such as real-time PCR, next-generation DNA sequencing methods and DNA biochip or DNA microarray, which have been gradually applied to the detection and quantification of exogenous DNA in food samples, even if present in small amounts.

Keywords: Authenticity. Fraud. Animal products. Species identification. DNA-based identification.

Resumo

O aumento do poder de compra da população, especialmente em países emergentes como o Brasil, foi seguido pelo crescente consumo de carnes, leites e seus derivados, assim como pela maior exigência por padrões de qualidade destes produtos. Os diferentes tipos de fraude podem comprometer a qualidade e ferir os direitos do consumidor, sendo relevante a aplicação de métodos mais sensíveis e específicos para investigação da autenticidade de gêneros alimentícios de origem animal. A substituição total ou parcial de carne, leite ou derivados, de outra espécie animal que não a declarada no rótulo dos produtos, compromete a natureza e a qualidade destes produtos, prejudicando os direitos de escolha dos consumidores, que podem estar relacionados a recomendações médicas e nutricionais, ao valor econômico do produto ou sobre os hábitos e/ou restrições alimentares específicos de cada cultura. A identificação das espécies animais que deram origem aos produtos cárneos e lácteos é importante para a rastreabilidade dos alimentos. Embora matrizes alimentares tenham composição complexa e variável, técnicas biomoleculares têm sido cada vez mais utilizadas para a identificação de espécies animais, uma vez que tem sido demonstrada a confiabilidade, especificidade, rapidez e alta sensibilidade, mesmo quando utilizadas em amostras mistas. Esta revisão teve como objetivo apresentar os principais métodos moleculares que podem ser utilizados para a detecção da adulteração de espécies em derivados cárneos e lácteos, incluindo métodos já bem estabelecidos, tais como a reação em cadeia da polimerase (PCR), bem como as tecnologias mais avançadas, como a PCR em tempo real, os métodos de sequenciamento de DNA de última geração e o *biochip* de DNA ou DNA *microarray.* Esses métodos moleculares vêm sendo utilizados, com sucesso, na detecção e quantificação de DNA exógeno em amostras de alimentos, mesmo que este DNA esteja presente em pequenas quantidades.

Palavras-chave: Autenticidade. Fraude. Produtos de origem animal. Identificação de espécies. Identificação baseada no DNA.

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Received: 22/02/2015
Approved: 29/07/2015

DOI: 10.11606/issn.1678-4456.v52i3p183-194

Introduction

Animal products stand out among the main food items of agribusiness chains, due to their high nutritional value. Data from the United Nations Food and Agriculture Organization (FAO), indicate a rapid livestock growth due to the growing demand for animal products like meat, milk and milk derivatives (SPEEDY, 2003). It is estimated that meat consumption worldwide will reach 300 million tons in 2020, an increase of approximately 30% in the past 20 years. Likewise, an increase is also expected in milk intake, from 568 to 700 million tons, which corresponds to a 25% increase over the same period (DELGADO et al., 1999). Brazil is one of the great powers of world agribusiness, standing out as a major producer and exporter of various agricultural genres (IBA et al., 2003). Beef is at the top of the food hierarchy, comprising approximately 49.6% of total meat consumption in Brazil, followed by poultry, at 34.6%. Pork, although it is the most consumed meat in the world, only represents 15.8% of total meat consumption in Brazil (THOMS et al., 2010). In the first decade of the 21st century, the country became the largest exporter, the second largest producer and third largest consumer of beef in the world (RIBEIRO, 2013). The production chain of beef circulates about US$ 167.5 billion per year. In 2012, Brazil exported beef to 142 countries and the gross value of production in 2013 was approximately R$ 51.1 billion (NEVES, 2012; BRAZIL, 2014).

However, when considering dairy products, Brazil has always been a traditional importer, since different factors affect Brazilian dairy quality, such as animals with inappropriate genetic potential and/or inadequate herd food, reproduction and the persistence of certain diseases eradicated by competitors but present in the national cattle herd, such as brucellosis and tuberculosis (FIGUEIREDO et al., 2008; BRAZIL, 2014; CARVALHO et al., 2014). As a consumer option, dairy products from goats have been consolidated as a profitable activity in the country: annual production is approximately 148,149 tons, with 90% of this total originating from the northeastern region of Brazil[1]. Despite these values, the challenges for national goat dairy industries can be associated with the process of obtaining the milk, in which the production per animal is less than for cattle, and with problems related to a market still under development, since consumers show reluctance to accept these dairy-derivatives, due to the characteristic taste and odor of the milk (WHETSTINE et al., 2003).

Considering the production of milk-derivatives, Brazil is the world's sixth largest cheese producer, although consumption is still considered modest in the country, with approximately 4.5 kg consumed *per capita*, while in Argentina the consumption is 11 kg *per capita* and in France, 23 kg *per capita* (CHALITA et al., 2009; AVANÇOS, 2011). Although most Brazilian cheese production is derived from cow milk, there is a growing market for cheese produced from the milk from other animal species, such as sheep, goats and buffalo (BORTOLETO; WILKINSON, 2000).

According to the United Nations data, world population reached 7.2 billion people and, according to forecasts, will exceed 9 billion by the middle of this century. With this population growth, the challenge of providing food in adequate quantities and quality has increased. The great demand for food can, in a way, encourage the practice of fraud in various foodstuffs (GALEAZZI et al., 2002). The addition of products without prior declaration on the label, besides representing fraud and deceiving the consumer, can also bring health risks (GOLINELLI, 2014).

Thus, new technologies are necessary to analyze food products in order to meet the needs of consumers and governmental regulatory agencies. The processing of animal products, especially heat treatment, may compromise the most commonly used authenticity tests, based on protein analyses, due to their denaturation at high temperatures. Alternatively,

[1] http://faostat.fao.org/site/339/default.aspx

the assessment of the authenticity of dairy and meat products can be performed by different methods based on DNA analysis, targeting the sequences of conserved genes present in the genome of each species. This new methodological approach for fraud detection in different foods has gradually improved and today those methods not only show greater reproducibility, precision and accuracy, but also reduce the time of the assay, allowing for a greater number of samples tested and tests that are easier to perform.

The aims of this review are to present and discuss the applicability of several molecular tools for the detection of adulteration and investigation of genetic traceability in animal products, especially meat, milk and dairy products. Herein, studies based on the most widespread tests will be presented, such as PCR (polymerase chain reaction) and next-generation DNA sequencing, formerly used in clinical research and now adapted for the detection of adulteration in food matrices.

Adulteration and authenticity of animal products – meat and milk derivatives

Fraud is a term that applies to any practice that is not universally accepted, to be applied without the consent of official regulatory agencies, leading to modifications of a food product, always for profit and disregarding consumer rights (KOLICHESKI, 1994). Fraud can be practiced at different levels, from the crude and easily recognizable to the most elusive and difficult to detect (EVANGELISTA, 1989). Fraud can generally be classified into four types: fraud by alteration, forgery, sophistication or adulteration. According to the National Health Foundation (*Fundação Nacional de Saúde* – FUNASA - Brazil) food adulteration can be defined as the contamination, deterioration or alteration of the nutritional properties of the foodstuff in question.

Food can be considered adulterated if it contains any foreign substance, when a substance has been removed beyond the tolerance limit, any component has been omitted or any color, preservative or substance not allowed by the current health legislation has been added (BRAZIL, 2004). The RIISPOA (Regulation of Industrial and Sanitary Inspection over Products of Animal Origin) considers food products as adulterated when altered or impure raw materials, different and/or containing material from different species, have been used in standard food composition without the prior authorization of the inspection department of products of animal origin and without the modifications being listed on product labels. It is noteworthy that tampering can also be related to the replacement of high-cost components by other, less expensive, ones (SILVA et al., 1999).

In relation to animal products, especially milk and meat, the adulterations include replacing more noble raw materials for other materials of inferior quality or lower market value, always aiming to increase profits by hurting consumer rights. This practice may compromise product competitiveness in the international market, bringing great losses to Brazil, constituting a serious and heinous crime, since the fraud or tampering of food products goes against public health practices, according to Federal law 9695, 1998 and the Brazilian penal Code, law No. 2848 of December 7, 1940 (BRAZIL, 1940).

In the broader social context, authenticity can be defined as what is believed or accepted to be true or real. It is related to the certainty that the product comes from sources with references, without being subject to changes, thus corresponding to the expectations associated with said product (LU; FINE, 1995). The fraudulent adulteration of food products can compromise consumer health, such as possible health damages caused by the ingestion of non-declared constituents in individuals with medical or nutritional constraints, such as allergies (MACKIE, 1996).

Food allergies are an important type of atopic disease, and cow milk proteins (casein, β-lactoglobulin, α-lactalbumin, serum albumin and globulin) are the major allergens in food matrices (MORAIS; SPERIDIÃO; SILLOS, 2013). Lactose intolerance is caused by primary or secondary deficiency of the enzyme responsible for lactose hydrolysis, namely lactase or β-galactosidase, preventing the hydrolysis of disaccharides to galactose and glucose, resulting in lactose malabsorption, which accumulates in the small intestine and may cause diarrhea and abdominal discomfort (SWALLOW; POULTER; HOLLOX, 2001). In other similar diseases, such as phenylketonuria and irritable bowel syndrome, the restriction of certain protein foods is recommended, including milk (ZEMAN; BAYER; ŠTĚPÁN, 1999; STAUDACHER ; PARKES, 2014).

To prevent calcium deficiency in individuals with dietary restrictions to cow milk, it is advisable to include milk obtained from other animal species in the diet (HAENLEIN, 2004). Goat milk has better digestibility, especially because it contains lower α-s1-casein content and higher percentages of short- and medium-chained fatty acids (ALBENZIO et al., 2012). Goat milk has higher amounts of calcium, iron, zinc, molybdenum and sulfur compared to cow milk, and its importance in infant feeding is not limited to the biological value of its nutrients, since it also possesses nutraceutical and hypoallergenic properties (YANGILAR, 2013). Another option to cow milk may be the consumption of buffalo milk, which shows similar high-nutritional value, high fat, protein and mineral levels and can be eaten either fresh or used as the raw material for producing derived dairy products, such as mozzarella cheese (AHMAD et al., 2013; GUIMARÃES, 2014). Sheep milk also has higher protein content compared to milk cow, goat and buffalo milk, its proteins are considered of high biological value and contain higher vitamin C and biotin concentrations than cow milk (BENCINI; PULINA, 1997; MAYER; FIECHTER, 2012; CLAEYS et al., 2014).

The search for quality products necessarily includes the authenticity of these foodstuffs. This results in increasing pressure on control agencies for the establishment of government food control policies during the different stages of the production chain and, most important, the increasingly rigorous labeling of food products in order to comply with consumer rights to choose a certain foodstuff, whether due to economic, health, or religious and cultural issues. Dietary restrictions are a part of many cultures and religions. A very severe restriction is the prohibition of pork intake in the Muslim and Jewish cultures, and in the latter, strict standards for slaughtering, preparation and consumption of meat must also be observed (ABU-SAAD et al., 2012). Cultural differences must be considered when determining consumption trends, especially regarding the acceptance and intake of meat from different animal species. The consumption of meat that is considered exotic by many cultures, such as dog meat, is common in countries such as Korea, China and Oceania, while horse meat is appreciated in France, Belgium and Japan (FISCHLER, 2001). Although still a nascent market, the commercialization of exotic meat in Brazil as a new consumption option has increased progressively, and frog, ostrich, wild boar, buffalo and alligator, among others, are now available in large consumer centers. However, the demand for exotic meat is still insignificant when compared to beef, since exotic meat is not appreciated by the majority of the population (SUZAN; GAMEIRO, 2007).

A major fraud in animal products is the adulteration of dairy products by adding cow's milk to milk obtained from other species, such as sheep, goats and buffalo. Since the cattle herd in Brazil is larger than the herds of these alternate species, milk production per animal in the latter is smaller and has seasonal variations, in addition to higher costs than cow milk (LOPEZ-CALLEJA et al., 2004). Thus, this fraudulent practice can hurt consumer rights, both due to economics and related to the risk of milk consumption, which is not declared on the label. Furthermore, it is important to determine the type of milk (animal species) used in cheese manufacturing to ensure the authenticity of the product (BRANCIARI et al., 2000; MAFRA et al., 2004).

As it occurs in dairy products, the adulteration of meat products is also practiced by partial or complete replacement by meat from species with low commercial value. The motivation for these fraudulent practices is mainly economic, but this can affect the health of individuals and communities, causing economic impact on public health, if the milk or meat of low allergenic potential is replaced by another with high allergenic potential.

In addition, as food security can be also related to the microbiological aspect, and different species can show species-specific microbiota, the consumption of different meats from species other than those declared on the label increases the risk of food poisoning by improper cooking processes (MAMIKOGLU, 2005; CAWTHORN; STEINMAN; HOFFMAN, 2013).

Main molecular methods used for the detection of dairy and meat product adulteration

The continued occurrence of food scandals, especially concerning animal products, coupled with economic and social reasons, has contributed to increased consumer interest in the food they eat and how it is produced.

Traceability allows food businesses to target products affected by food safety problems, minimizing disruption to trade and any potential public health risks.

Traceability can be applied to give information about animal species, origin and production system. The genetic traceability is based on the identification of both animals and their products through the analysis of DNA sequences (DALVIT; DE MARCHI; CASSANDRO, 2007).

Following the determination of the DNA structure by Watson and Crick (1953), several studies with different goals have been conducted, initially in clinical research and progressively in other areas, such as the food sciences, to evaluate the authenticity of food from animal origin. Tests based on DNA sequences are applied in the detection of species adulteration, because this molecule is present in every cell of the organism, is conserved during animal life and variable among individuals (CUNNINGHAM; MEGHEN, 2001).

The mitochondrial DNA (mtDNA) possesses several advantages over nuclear DNA for studies about speciation in animal products. mtDNA is found in considerable amounts (millions of copies) in every cell; tends to be maternally inherited so that individuals normally possess only one allele; possesses relatively high mutation rate compared to nuclear genes with accumulation of enough point mutations to allow the discrimination of even closely-related species. Thus, these differences may positively affect test sensitivity (LOCKLEY; BARDSLEY, 2000).

It is important to consider that DNA-based methods are highly dependent on DNA extraction and purification techniques. In particular, DNA preparation from food matrices requires stringent extraction and purification strategies that ensure efficient recovery of nucleic acid and removal of the numerous compounds inhibiting PCR assay. Different methods were evaluated, as organic extraction, with a variable loss of amounts of the original sample, and the commercial kits. While many kits have been developed to assess clinical specimens, they have been adapted for analysis of food matrices. These extraction kits, although more expensive, are usually more efficient (DI PINTO et al., 2007).

In 1985, Kary Mullis developed the polymerase chain reaction (PCR), which is a simple, rapid, sensitive and specific tool for the analysis of nucleic acids. The test is based on the exponential amplification of a DNA sequence in order to enable millions of copies of a specific nucleotide segment, obtained by catalysis of the Taq DNA polymerase enzyme and a specific set of primers targeting the sequence in the adulterant genome of the species (FAJARDO et al., 2007). Since then, several PCR-based methods have been developed and used for the detection of animal origin constituents of in food, such as nested and multiplex PCR, PCR-RFLP and real-time PCR.

The main aim of the nested PCR technique is to increase the specificity of the reaction, in which an amplification step using one set of primers is performed with several cycles. The amplification product is again re-amplified using another set of primers based on a sequence contained within the first set of amplified primers (UNAJAK, 2001). The multiplex assay allows for the simultaneous identification of various animal species, more quickly and less expensively, in which more than one pair of primers are used (GHOVVATI et al., 2009; KÖPPEL; ZIMMERLI; BREITENMOSER, 2009; BROLL, 2010).

The restriction fragment length polymorphism (RFLP) technique associated with PCR, or PCR-RFLP, displays great specificity and has been widely used in the authenticity evaluation of dairy products (VERKAAR et al., 2002). The pattern of restriction fragments is commonly used for genome mapping, localization of genes for identification of genetic disorders, determination of risks for certain diseases and paternity testing, and was adapted to compare DNA from food samples with those obtained from each animal species, where similar patterns indicate the presence of such species in food matrices. Real-time PCR, as described by Higuchi et al. (1993), is a more sophisticated tool than conventional PCR and allows for the identification of species in a food matrix, even if the product is highly processed. The fluorescence detection system enables simultaneous reproducible, precise and sensitive detection, quantification and amplification of DNA in a single step, identifying and quantifying derived products from different animal species. The most widely used fluorescent compounds are SYBR-Green and the TaqMan® probe, the latter adding greater specificity to the assay (KESMEN et al., 2009). Quantitative information helps establish the boundary between the permissible and non-permissible limits of contamination, if the adulteration is deliberate or unintentional of certain species in processed foods (DI PINTO et al., 2007).

The association of PCR followed by the sequencing of the amplified genes or gene sequences with high diversity among animals allows for the identification of animal-derived species in complex food matrices, identifying whether there are species present in the composition of food that were not declared on the product label. Gene sequences present in the mitochondrial genome of animals have been used as a target for the unambiguous identification of the species, since they present more interspecies genetic diversity, but are highly conserved between individuals of the same species (intraspecies). The identification of species related to each other, such as *Bubalus bubalis* and *Bos taurus*, commonly used in the investigation of buffalo mozzarella tampering with cow milk, is based on the mitochondrial cytochrome b gene (cytb) and the 12S rRNA subunit (NAU et al., 2009), generally allowing for the identification of the contaminant species (FAJARDO et al., 2008; BOTTERO; DALMASSO, 2011).

Marketed beginning in 2005, next-generation DNA sequencing technologies have evolved rapidly and are considered very promising for fraud investigation of different food matrices. These tools sequence DNA in platforms capable of generating information on sequences of millions of base pairs in a single sequencing cycle, with low costs and in less time than other available techniques. Among the new sequencing platforms, the Roche 454 FLX and Solexa Illumina are noteworthy, as well as the *SOLiD System* platform from Applied Biosystems. The Ion Torrent platform enables the complete sequencing of small genomes and transcriptomes. Thus, the simultaneous DNA sequencing of multiple samples and detection of different animal species through semiconductor chip technology captures chemical reaction sequencing signals and converts them to the base calling information (DALLOUL et al., 2010; TABERLET et al., 2012; VARUZZA, 2013).

Different markers have been studied for adulteration detection and the most widely used are microsatellites also known as short tandem repeats (STR) and single nucleotide polymorphism (SNP) (MARIANI et al., 2005). Another modern approach is the technology of DNA biochip or DNA microarray that allows the examination of complex mixtures of PCR products and has potential to simultaneously identify hundreds or even thousands of species. The technique involves the amplification of a small segment of mitochondrial gene with a labeled fluorescent dye using a pair of primers that target a conserved sequence of closely related species. Basically, two types of species-specific DNA chips for meat authentication are available in the market, Chipron LCD array kit (Chipron, Germany) and oCheck® detection system (Greiner Bio-One). Both are based on mitochondrial 16S rRNA gene and allow simultaneous detection of up to 14 species and 8 species, respectively, within 3 hours (DI PINTO et al., 2007).

In addition to the DNA-based methodologies for food authentication, some analytical methods based on the detection of specific proteins expressed by certain animal species are also available, detected by chromatographic methods, such as HPLC and GCMS, electrophoresis, such as denaturing SDS-PAGE, and enzymatic ELISA-based immunoassays (REID; DONNELL; DOWNEY, 2006; MONTOWSKA; POSPIECH, 2010). Although the set of expressed proteins can unequivocally identify the species of an adulterant organism, DNA, however, is a tridimensional structure that has higher chemical stability when compared to protein molecules, and is resistant to heat treatment, which meat and dairy products usually undergo (DALMASSO et al., 2004).

As mentioned previously, several methods based on biological, chemical or sensory markers of authenticity can be used to assess the authenticity of food products, especially meat and dairy-derived products, screening food manufacturing from raw material to finished product.

Literature Databases - fraud in animal products

In a brief review, some studies regarding the tampering of animal products were selected. Indexed publications on the Medical Literature Analysis and Retrieval System Online (MEDLINE) databases, queried by the PubMed; Cochrane and Scientific Electronic Library Online (SciELO) were obtained. The following Boolean operators were used ***"food"*** *AND* ***"animal"*** *AND* ***"adulteration."*** Eligible, complete articles published from 1994 to 2014, in the English language, were considered. The screening process evaluated the titles and abstracts in a search conducted in July 2015, resulting in 177 selected publications. When the search was reset with the terms ***meat*** *and* ***adulteration*** *or* ***fraud***, 166 references were recovered, of which 71.1% were published in the last 10 years. Among the selected references, studies related to the adulteration of meat products with distinct animal species from what is specified on the product label are noteworthy. This type of fraud was detected by different methodologies based on DNA sequencing, primarily by PCR and its variations, and the partial sequencing of nuclear DNA targeting the 16S rDNA (or 12S rDNA) or mitochondrial DNA (DALMASSO et al., 2011; DE et al., 2011; RODRIGUES et al., 2012; NEJAD et al., 2014; XU et al., 2014).

Among the foodstuffs cited for tampering are *frescal*, matured or cooked, restructured and packed products. Ghovvati et al. (2009) observed that 40% of sausage samples and 30% of other types of cold-cut samples contained poultry residue. Hsieh et al. (1996) identified the presence of adulterant species in 54% of fresh sausages of 87 analyzed samples, which were not stated on the product labels. Horse meat was identified in 09 of 23 hamburger samples and 02 of 17 Mexican sausage (chorizo) samples (FLORES-MUNGUIA; BERMUDEZ-ALMADA; VÁZQUEZ-MORENO, 2000). A pilot study conducted by the Food Safety Authority of Ireland (FSAI) showed unreported horse meat in 03 of 24 packed samples. When investigated by the Irish government, the complexity of the supply chain prevented the precise traceability of the point at which the tampering occurred (O'MAHONY, 2013).

The Food Standards Agency of the United Kingdom (FSA) showed that 10 of 27 beef hamburger samples contained horse meat (FSA, 2013). The more recent scandal in early 2014 of the adulteration of meat and meat products with horse meat attracted the attention of food producers and the retail market, highlighting the need for a more elaborate traceability system regarding the production chain by the European Union. The provisions of Regulation No 931/2011 of the European Union were passed into law in the case of foodstuffs of animal origin (MOHAMMED; ISMAIL; CAVUS, 2014).

Seventy-seven studies were found when conducting a search at the PubMed database with the terms ***milk*** *and* ***adulteration*** *or* ***fraud***, for the same period and language, with an added filter for other animal species. These include the adulteration of milk products, such as tampered buffalo mozzarella cheese and goat and sheep cheese with cow milk (MININNI et al., 2009; SOARES et al., 2010; COTTENET; BLANCPAIN; GOLAY, 2011). Czerwenka, Műlller and Lindner (2010) detected tampering in 03 of 18 of buffalo mozzarella samples, showing high content of cow milk (11%, 66% and 87%). In Romania in 2010 67.3 and 79.7% of 73 sheep milk and goat cheese samples, respectively, showed the presence of cow milk (STĂNCIUC; RÂPEANU, 2010). Golinelli et al. (2014) reported the fraudulent addition of cow milk in all goat cheese brands marketed in the city of Rio de Janeiro. *Frescal* (fresh) goat cheese adulteration was evaluated by PCR, combining the cheese composition analysis and sensory perception of tampering by consumers. 45% of the consumers, approximately 46 panelists, were able to perceive amounts up to 10% cow milk replacement in goat milk.

In the retrieved studies, fraud was detected by methods based on DNA analysis, mainly by PCR and its variations, and also by partial sequencing of nuclear (16S rDNA or 12S rDNA) or mitochondrial DNA (DALMASSO et al., 2011; DE et al., 2011; RODRIGUES et al., 2012; NEJAD et al., 2014; XU et al., 2014).

Final comments

Brazil, with the largest herd of beef cattle in the world, stands out in the international arena as the main beef exporter to EU countries. Therefore, pressure by the 26 countries comprising the EU that import beef from Brazil has increased the need for safe and fraudulent-free products. The present study suggests that the molecular techniques based on DNA analysis, adapted to the evaluation of the authenticity of complex and highly processed food matrices, should be incorporated into routine analyses by the regulatory and supervisory food security agencies.

Although the tools based on DNA analysis are not yet routinely used by governmental agencies responsible for investigating fraud in foods, especially in dairy products and meat, their use is justified by both economic aspects, because of the high consumption of these products, and legal aspects, regarding violation of consumer rights, as well as meeting the demand for safe products for international and domestic markets. Therefore, more sophisticated and fast methods must be used to investigate the authenticity of food matrices of animal origin.

Methodologies considered the gold standard for researching the authenticity of animal products have not yet been established in Brazil. Molecular tests based on PCR and its variations, associated or not to sequencing (including third and fourth generation technologies) are increasingly emerging as a more efficient alternative to evaluate a large number of samples and processed foods from different food matrices. The sequencing of mitochondrial genes is a sensitive and specific tool, which allows clear and especially fast identification (in about two days) of contaminant(s) regardless of the amount present. Generally, these molecular tools have a higher rate of cost effectiveness, at medium and long term, since they show greater accuracy and reproducibility, allowing for faster analysis and with a greater number of samples analyzed in a single assay, while also forcing a more responsible attitude among producers and food industries concerning label products and the replacement of adulterant species in food from animal origin.

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