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Review Article

# Molecular Techniques for identification applied to food: A review

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## Abstract

This review aims to address the use of molecular techniques applied to the identification of food. The application of molecular techniques in food is large, several authors comment on the detection and quantification of strains of pathogenic microorganisms present in foods and the presence of toxins and waste allergens not related to specific goods in production lines. They are also used to control the authenticity of many foods, very important for receiving raw material industries, identification of genetically modified organisms, and genetic selection in yeast for fermentation processes. The methods presented in this review proved to be fast, and effective and could be effective substitutes for chemical and physical methods, chemical and biochemical, producing results with greater accuracy, reliability and speed of analysis.

## Introduction

The growing food fraud issues have enforced the development of food testing not only at the scientific research level but also at the industrial and enforcement levels, to detect manufacturers' misconduct [1]. Beef, buffalo, chicken, duck, goat, sheep, and pork are heavily consumed meats bearing nutritional, economic, and cultural/religious importance and are often found to be mutually adulterated in raw and processed states [2].

Another problem is the involuntary cross-contamination that can promote adulteration of products or contaminating agents caused by sharing equipment between different plant and animal species or due to unhygienic human handling practices [3].

For a long time, food analysis has always been based on physical, chemical, physicochemical, and biochemical methods, however, these are techniques that sometimes require a lot of time and have a low degree of precision. Molecular techniques have facilitated the identification, genotyping, and monitoring of food contamination sources, identification of genetically modified organisms, and use as food detection methods to avoid falsification through genetic markers.

Instrumental analyses, commonly used in physical-chemical methods, present systematic errors related to the equipment and reagents used, as well as errors in reading, and weighing by the operator, and require several hours before the results are obtained.

A variety of immunoassays are increasingly applied in food safety because they are fast, simple, and cheap [4]. According to [5] the results of biochemical tests used for bacterial identification and bio-typing may show variability due to the action of environmental factors on gene expression, the low discriminatory power of microorganisms with little genetic variability, and the risk of erroneous interpretations.

The trends are the development of diagnostic devices for the rapid identification of target analytes [6] such as allergenic compounds in food, food contaminants, chemical additives, veterinary drugs, heavy metals, and other substances of interest to the food industry and consumers.

Studies have been demonstrating the effectiveness of molecular techniques regarding the accuracy of the protocols



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used and the agility to provide the necessary answers. The main authors of the theme defend the use of these techniques to improve the quality assurance of foods, in addition to studying the molecular mechanisms of the main contaminants.

Immunoassay refers to a class of analytical methods based on antigen-antibody-specific interactions. Traditional labels (such as an enzyme, colloidal gold, and chemical luminescence agent) are combined with antibodies or antigens to prepare probes. The signal intensity from probes could reflect the content of targets after the probes specifically interact with targets in the given sample. There are two formats in each immunoassay test: 1) sandwich format (for macromolecule detection, such as foodborne pathogens and allergen proteins). 2) Competitive format (for small molecule detection, such as pesticides, antibiotics, mycotoxins, etc.) [7].

In general, the most known and used methods are Western Blots (WB), Enzyme-Linked Immune Sorbent Assay (ELISA), Lateral-Flow Assay (LFA), and Dot-Blot Assay (DBA) and have been applied in the detection of carcinogenic compounds, pathogenic and allergenic microorganisms using antibodies [8].

The objective of this review article was to present the main molecular techniques for identification and their application in food.

## Western blot

It is a molecular biology method for the detection of proteins using gel electrophoresis for the separation of proteins according to their size to be identified. Samples may come from plant tissue extracts or cell cultures. Sample preparation for this test consists of breaking down plant tissue or cell culture to release the proteins of interest where they are solubilized so that the proteins can migrate individually through a separation gel. Protease and phosphatase inhibitors must be added so that the proteins remain intact [9].

Electrophoresis is an analytical process used for the separation of electrically charged particles, which occurs when they are dissolved or suspended in an electrolyte, through which an electric current is applied. It can be one-dimensional, which has only one plane of separation, separating them by their isoelectric point (commonly used for routine protein and nucleic acid separation), or two-dimensional, which has two planes of separation, separating them by their molecular weights and is used for "fingerprint" analyses, generating results with greater precision and high resolution of the proteins present in the cell [10].

To make the proteins accessible to detection, they are moved from the gel onto a nitrocellulose or PVDF membrane. The membrane is placed in contact with the gel and an electric current is applied between the plates so that the charged proteins move from the gel to the membrane while maintaining the arrangement. The result of this process is called "blotting", where the proteins are exposed to a thin layer for detection. The binding between proteins and the membrane is based on hydrophobic interactions and interactions between charges [11].

During the detection process, the membrane is tested with antibodies to the protein of interest, and it binds with a revealing enzyme, which causes a color change or emits a photometric signal. Colorimetric detection relies on incubating the Western blot with a substrate that reacts with the revealing enzyme that is bound to the secondary antibody. This step will convert the soluble dye into an insoluble form of different colors that will precipitate next to the enzyme, coloring the membrane. Stain development is stopped by washing out the soluble dye. Protein levels are evaluated by densitometry or spectrophotometry. The literature mentions that other detection methods such as chemiluminescence and radioactive and fluorescent detection can be used [12,13].

According to [14], the "Western Blot" has the potential to overcome some of the main problems associated with immunoenzymatic detection of toxins present in foods, such as cross-reactivity with unrelated antigens and insensitivity with food heat treatment. The authors developed a protocol to measure and identify the level of Staphylococcus aureus enterotoxin. The results obtained suggest that the "Western Blot" is a useful tool for determining the presence of enterotoxins from Staphylococcus aureus because it allows the characterization of the antigen and the antibody reaction and can be used for foods submitted to heat treatments, thus overcoming some limitations.

[15] studied polyacrylamide gel electrophoresis and SDS-PAGE with loading into gel wells using acid dyes (tartrazine, brilliant blue, or new cocaine), which allowed easy visualization of stacking gel wells and direct loading of samples into these wells, facilitating protein detection.

Since sensitization to a specific food allergen does not occur only through oral exposure, but also through skin contact or inhalation [16]. In this sense [17], sought to detect silkworm allergenic compounds in humans from western blotting of moth wing extract with sera from patients sensitive to silkworms for the identification of proteins.

## Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Polymorphism (RFLP) is based on the hydrolysis of DNA by a restriction enzyme, exploring variations in homologous sequences of this DNA. The procedure begins with DNA extraction and is followed by restriction enzyme digestion. The separation of the fragments is done by size through electrophoresis, later, the fragments are transferred to a nylon membrane where the hybridization between the fragments and the marker probes occurs, determining the length of the fragments that are complementary to the probe. An RFLP occurs when the length of a detected fragment varies between individuals [18,19].

[20] analyzed the mitochondrial DNA by RFLP for the differentiation of fish species because the falsification of some species is very common, such as salmon, for example. They developed a method of amplification of a specific part of mitochondrial DNA and used RFLP to distinguish between them. The amplifications were hydrolyzed by the enzyme



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restriction endonuclease (RE), resulting in the polymorphism of restriction fragments, and from this, it was possible to identify the different species of fish, a tool that can be used in receiving raw material from numerous processing industries of fish to ensure that the product they are receiving matches the supplier's specifications.

The adulteration of spelled (hexaploidy wheat species) has been a problem in many European countries such as Germany, Austria, and Switzerland, due to its small cultivation, which makes its price high, many suppliers mix spelled grains with wheat grains. Because they are morphologically similar, they go unnoticed in the quality control of many industries. Due to this problem [21], studied the gamma-gliadin gene polymorphism of spelled wheat as a tool for authenticity control. They concluded that the RFLP-LOC-CE was a perfect analysis for the quality control of cereals, seeds, and pure cultivars.

Detection of fragmented DNA by microgel electrophoresis in single cells or nuclei has shown good results, but the test is limited to untreated foods as this results in fragmentation [22]. The author comments on the use of RFLP as a mechanism for detecting irradiated foods, where irradiation is applied as a microbial reduction treatment. no to this process.

The differentiation of cattle species in beef by mitochondrial and satellite DNA RFLP was studied by [23], where they describe two methods for the identification of bovine species. The first method is based on species-specific mutations in the DNA of cytochrome-b and cytochrome oxidase II, the second, we used RFLP as a confirmation method on satellite DNA. All bovine species analyzed could be identified. The authors state that all relevant reference animals should be analyzed in parallel and that differentiation should be based on at least two enzyme restriction sites in order to exclude intraspecies polymorphism.

The identification of wine yeasts by RFLP without prior plate isolation was tested [24]. In this study, species of yeast collected from a must sample during the wine fermentation process were identified, and amplification was performed by PCR and RFLP, analyzing the purified DNA directly from the sample, without the isolation of yeast in plaque. The authors concluded that the proposed method is effective in characterizing the yeast population and detecting undesirable yeasts, such as deteriorating yeasts present in the initial phase of the wine fermentation process. However, the authors suggest additional studies to evaluate the reproducibility of the method.

In this case, we have the use of RFLP as a tool for optimizing beverage fermentation processes, where it can promote the improvement of yeast cultures used in these processes, accelerating the process and avoiding the production of undesirable products.

[25] studied the co-amplification and sequencing of a cytochrome-b fragment that affects cattle identification in RFLP food authentication studies. The results obtained in this study concluded that authentication studies by RFLP analysis can be complicated in the case of cattle by the co-amplification method of two distinct cytochrome-b fragments. Primers aimed at the mitochondrial DNA sequence allowed the discovery of an RFLP pattern complex in cows of local breeds from northern Spain.

The use of RFLP for the identification of species used in commercial Jerky Beef was studied by [26], where they based the study on the analysis of the mitochondrial 12S rDNA gene. Seventeen samples of animal tissues and eleven commercial products of Jerky Beef were used, as a conclusion, the authors state that the method used was efficient for the authentication of products from five animals.

[27] used the heptalex polymerase chain reaction-restriction fragment length polymorphism assay for the detection of edible animal species. Target specificity was confirmed by cross-amplification reaction with 25 non-target species and PCR products were authenticated by enzymatic digestion. The method was considered sensitive and specific, with a detection limit of 0.5%.

## Pulsed Field Gel Electrophoresis (PFGE)

Pulsed-field gel electrophoresis was developed by [28] and consists of genomic macro description, through the use of restriction enzymes with low cutting frequency, such as SmαI, AscI, and ApαI, followed by electrophoresis. in agarose gel where the orientation of the electric field that travels through the gel is periodically modified. And it is due to this variation of the electric field in the electrophoresis system that it is possible to separate large DNA fragments of sizes greater than 1 Mb.

When working with DNA fragments larger than 500 kb, it is necessary to protect these molecules from mechanical breakage and keep the DNA intact during the extraction process. For this, in the case of bacterial DNA samples, they must be soaked in an agarose gel that protects them from breakage but allows the free flow of the necessary solutions for the lysis and digestion of the cell and the DNA [29].

[30] verified the genetic and antigenic diversity of strains of *L. monocytogenes* isolated in a gravlax salmon production line and correlated the genetic diversity of the strains with their distribution along the processing line. The author used one hundred and eighty-one strains of *L. monocytogenes* from samples of gravlax salmon collected at different stages of processing, sample handlers, environments, and utensils. The strains were typed using the PFGE technique. With the results obtained, the author concluded that there was a predominance of strains belonging to serogroups 1 and 4, the latter, in the final product, is indicative of risk to the population consuming "gravlax" salmon. The raw material already arrives at the industry contaminated by subtype strains that are capable of surviving the various stages of processing and the environment, utensils and handlers can lead to contamination by *L. monocytogenes* of "gravlax" salmon through cross-contamination.

The use of PFGE when receiving the raw material, in this case, is necessary, considering that the author found the presence of strains before processing, thus, PFGE will be of



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vital importance for the evaluation of the quality of the material maid. Interpretation of chromosomal DNA restriction patterns produced by PFGE and criteria for bacterial typing were discussed by [31]. PFGE has permitted to resolve of the intact chromosomal DNA from unicellular eukaryotes like fungi, yeast, and parasites in band patterns named electrophoretic karyotypes [32].

[33] commented on the various applications of PFGE. They cite that the method provided a new analysis mechanism for bacterial genomes, using different enzymes, being a powerful technique for the rapid resolution of this type of genome into a small number of large DNA fragments. This technique is useful for establishing the degree of relatedness between different strains of the same species. PFGE has shown utility in the study of radiation-induced DNA damage.

[34] evaluated the fingerprints of Salmonella enterica strains in food of animal origin in the city of Riyadh in Saudi Arabia using the PFGE method. In all, thirty-seven strains of subspecies of Samonella enterica were isolated from samples of chicken, which is the main host of Salmonella infection. The samples revealed a high degree of identity and similarity (< 99%).

## Polymerase chain Reaction (PCR)

The polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify single copies or fragments of DNA by several orders of magnitude. PCR is a powerful technique that involves the in vitro enzymatic synthesis of millions of copies of a specific segment of DNA in the presence of the enzyme DNA polymerase. The reaction is based on the annealing and enzymatic extension of a pair of oligonucleotides used as primers, which delimit the double-stranded DNA sequence targeted for amplification. These primers are artificially synthesized so that their nucleotide sequences are complementary to the specific sequences that flank the target region [35].

A PCR cycle involves 3 steps: denaturation, annealing, and extension. The target DNA double strand is denatured by raising the temperature to 92 °C to 95 °C. In the annealing step, the temperature is quickly reduced to 35 °C to 60 °C, depending essentially on the size and sequence of the primer used, allowing the DNA-DNA hybridization of each "primer" with the complementary sequences that flank the target region. Then, the temperature is raised to 72 °C so that the DNA polymerase enzyme carries out the extension from each 3' end of the primers. This extension involves the addition of nucleotides using the target sequence as a template so that a copy of this sequence is made in the process. This cycle is repeated a few dozen times. Since the amount of target sequence DNA doubles with each cycle, amplification follows a geometric progression such that, after just 20 cycles, more than a million times the initial amount of target sequence is produced. This amplification scale, therefore, allows starting with minimal amounts of DNA (on the order of a few picograms or nanograms) and ending the reaction with large amounts of DNA of a specific sequence of interest. The ease, speed, versatility, and sensitivity of PCR make it particularly powerful for molecular-genetic studies involving large numbers of individuals of any living organism [36].

[37] used the PCR technique to identify soy in meat products. The presence of soybean DNA was determined from soybean protein concentrates and determined with specific pairs of oligonucleotides. The test detected soy levels of 1%, which was confirmed by commercial enzyme immunoassay [38]. Discusses a universal PCR protocol for thirteen foodborne pathogens.

The use of PCR based on 16S rDNA for the identification of Clostridium perfringens in foods was studied by [39]. The PCR indicators were made by GenBank and are complementary only with the 16S rRNA gene, the test evaluated eleven strains of C. perfringens and thirty-eight strains of other species of bacteria, and the authors concluded that the method can be used in the identification of this microorganism in contaminated food, samples of one hundred grams of coxinhas inoculated with twenty, two hundred and two thousand cells of C. perfringens were submitted to the PCR assay and all results were positive.

The detection of the allergenic potential of residues of hazelnuts (Corylus avellana) in food was studied by [40,41] Studied the development of a PCR protocol to detect aflatoxins in food. In the present work, a reliable conventional method for PCR detection of aflatoxin templates from various species was developed. Fifty-six aflatoxin-producing strains commonly reported in foodstuffs were tested. With just the AFF1-AFR3 designed primer pair, the expected PCR product was obtained in all aflatoxin strains tested from various species and genera. This PCR protocol can be used as a routine technique to detect aflatoxin in food.

Traditional methods of cloning, sequencing, and DNA analysis were optimized or replaced by derivations of the PCR technique, we can mention as one of them the RAPD technique (random amplification of polymorphic DNA). It is a type of PCR, but the DNA segments that are amplified are random. When performing RAPD, several short primers of arbitrary sequences containing between 8 to 12 nucleotides are created, then PCR continues using a large genomic DNA template so that the fragments produced by RAPD are amplified. Unlike PCR analysis, RAPD does not require any knowledge of the target organism's DNA.

[42] analyzed Campylobacter spp. from environments and food [43]. Studied the genetic variability of *L. monocytogenes* from food isolates using RAPD-PCR [44]. Used RAPD-PCR to analyze the genetic diversity of fungi of the genus Trichoderma spp.

[45] studied the occurrence and dissemination of L. monocytogenes in a fresh mixed sausage processing plant, being evaluated by serology and RAPD-PCR. Strains biochemically characterized as L. monocytogenes were subtyped by serotyping. The strains were isolated in 25% of the samples, of which 94.3% belong to serogroup 1 and 5.7% to serogroup 4. It was observed that the occurrence and



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dissemination of L. monocytogenes in the processing plant studied are due to contamination through the equipment and processing environment.

A pretreatment method based on in situ enrichment culture with immunomagnetic separation combined with PCR for the rapid detection of *Salmonella* traces in milk samples was studied by [46] having shown a low detection limit and high specificity.

## Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Reverse transcription polymerase chain reaction is a variant of PCR, where one strand of RNA is reverse transcribed into its complement DNA via the reserve transcriptase enzyme. The resulting cDNA is amplified by PCR. This technique allows the detection of low levels of gene expression and facilitates the construction of cDNA libraries or the cloning of specific cDNA [47].

RT-PCR is sometimes confused with RT-PCR (real-time PCR), the latter revolutionized the process of quantification and fragmentation of DNA and RNA, performing quantification of these nucleic acids precisely and with better reproducibility, as it determines the values during the exponential phase of the reaction [48].

Studies using RT-PCR and real-time PCR for rapid detection and quantification of viable yeasts and contaminants in yogurts and pasteurized foods were developed by [49] Universal primers were designed based on fungal action sequences and were amplified by real-time PCR with a 353 bp fragment specific from the fungal species involved in food spoilage. From experiences with the heat treatment of food, actin mRNA was used as an indicator of cell viability, being effective, according to the authors. They concluded that the specificity and sensitivity of the procedure, combined with its speed, its reliability, and the technique's potential automation, offer several advantages for routine analysis programs that assess the presence and viability of fungi in food products.

[50] used real-time PCR for the detection of *Salmonella* in food. The work analyzed one hundred and ten food samples such as chicken, meat, fish, and raw milk. The diagnostic accuracy was 100% when compared to the traditional culture method. The overall analysis time of the PCR method was 24 hours, as opposed to the four or five days of the analysis time of the traditional culture method. Therefore, the authors concluded that the real-time PCR methodology can contribute to satisfying the great demand of microbiological quality control laboratories for agility in issuing reports about analyzed samples.

[51] studied the use of real-time PCR in the identification of L. monocytogenes in fortified food samples, the method was based on the ssrA gene, as a new diagnostic target.

The RT-PCR technique was used to detect sapovirus, which causes gastroenteritis in humans. The virus has the ability to contaminate edible shellfish. Thus [52], identified a set of primers for the detection of sapovirus with high specificity and sensitivity in food samples.

## Nested Polymerase Chain Reaction (Nested PCR)

This method is a modification of traditional PCR intended to reduce contamination of generated products due to the amplification of unexpected primer binding sites. The presence of PCR inhibitors in complex samples, such as food, can inhibit amplification and limit the usefulness of the technique.

The use of Nested-PCR for the identification of *Vibrio vulnificus* in fish, guts, and water was studied by [53].

The detection of *Shigella sp.* in foods by Nested-PCR was studied by [54], the method proposed by the author, according to him, has the potential to improve the detection of these microorganisms in foods, the protocol, which includes the combination of enrichment with the centrifugation of the floating density and the PCR assay offers better sensitivity and faster analysis than traditional culture methods.

A sensitive method for the detection of genetically modified organisms in maize using Nested-PCR was studied by [55].

[56] studied the comparison between DNA extraction and identification methods for Yersinia enterocolitica in meat products by Nested-PCR. The study compared three methods and concluded that the protocols were able to satisfactorily eliminate the PCR inhibitors present in food. The Nested-PCR test could be used effectively in the investigation of the pathogenicity of *Y. enterocolitica* in foods in the presence of interferents.

[57] used the nested PCR technique to investigate five nuclear genes regarding their potential application as specific markers for pistachio, an allergenic and potentially dangerous food for sensitive consumers. The efficiency of the technique was evaluated in wheat materials (dough) and ice cream, presenting validation regarding precision and veracity.

## Multiplex Polymerase Chain Reaction (Multiplex PCR)

Another test is based on modifying PCR in order to quickly detect deletions or duplications in a large gene. This process amplifies DNA genomes using various primers. Multiplex-PCR consists of multiple primer sets within a single PCR mix to produce amplifications of varying sizes that are specific for different DNA sequences. By targeting multiple genes at the same time, additional information can be obtained from a single test that would otherwise require multiple times the reagents and longer to run [58].

The annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction and size, i.e., their base pair length must be different enough to form distinct bands when visualized by gel electrophoresis [59].

Commercial multiplexing PCR kits are available and used by many forensic laboratories to amplify degraded DNA samples. Commercial kits have several advantages over laboratory



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multiplexing methods. Quality control measures are performed by the kit manufacturer and ensure that reactions are uniform across all kits. This avoids the preparation of PCR mixes that require laboratory skills and the use of multiple test tubes, increasing the risk of operator error and contamination [60].

This increased reliability allows profiles obtained from commercial kits to be admitted into court which is critical in major criminal trials. The use of specific kits across several laboratories also allows profiling results to be compared.

[61] studied the simultaneous identification of Escherichia coli serotype O:157:H7 strains and their toxin through Multiplex-PCR. The authors analyzed one hundred and eight bacteria; all strains being identified simultaneously with the types of toxins encoded by the strains.

The detection of pathogenic microbiota in shellfish using Multiplex-PCR was studied by [62].

Multiplex-PCR assay for the detection and identification of types of *Clostridium botulinum* A, B, E, and F in food was carried out [63]. The method employed four primers with equal melting temperatures, each one being specific for *botulinum* neurotoxin A, B, E, and F, allowing simultaneous detection of the four serotypes. A total of forty-three strains of C. *botulinum* and eighteen strains of other bacterial species were tested. Of seventy-two natural food samples investigated, two samples contained toxin B and one sample contained toxin E. The authors concluded that the assay is sensitive, and specific, and provides a marked improvement in the diagnosis of C. *botulinum* PCR.

[64] used real-time Multiplex-PCR for the detection and quantification of the 35S-CaMV promoter in genetically modified foods containing maize.

[65] developed a "seven-target" Multiplex-PCR for the simultaneous detection of transgenic soy and corn in feed and food products. The problem involves the increase in the number of genetically modified organisms (GMOs) authorized by the European Union, therefore, there is a need for methods that allow the rapid tracking of these organisms. The authors proposed a method to screen four transgenic maize species and one transgenic soybean species, for the implementation of rapid routine analyses. DNA was extracted from the corn and soybean samples and the recombinant DNA sequences were detected by seven pairs of primers with precision to be highly specific for each GMO to be investigated. In view of the results obtained, the authors concluded that Multiplex-PCR represents a fast and reliable screening method, being directly applicable in laboratories involving corn and soy raw materials.

The differentiation between the various serotypes of *L. monocytogenes* by Multiplex-PCR was studied by [66] where a new Multiplex-PCR assay was developed to separate the four main serotypes of the bacteria, which were isolated from food. The test has been successfully evaluated on two hundred and twenty-two listeria strains.

[67] studied through Multiplex-PCR the detection of *Campylobacter* spp. and *Salmonella* spp. in chicken meat. The study used specific primers for both microorganisms and fifty samples of chicken meat were analyzed 4% showed contamination by *Salmonella* spp. and 56% were contaminated by *Campylobacter* spp. The results obtained were confirmed by means of conventional culture methods. The method developed through Multiplex-PCR is relatively cheap and efficient for the detection of these bacteria after 24h.

Multiplex-PCR optimization for the simultaneous detection of bacterial spoilage agents in Iranian bread was studied by [68]. After inoculation of bread doughs with Bacillus licheniformis and Bacillus subtilis, DNA was extracted from the dough and subjected to PCR and then multiplexing. In conclusion, the authors state that this method offers greater accuracy and test speed, which are crucial criteria when dealing with food safety and large sampling volumes when compared to culture methods.

[69] investigated a multiplex PCR approach for the identification of Salmonella subspecies in chickens. The system showed low sensitivity and was able to distinguish 33 different subspecies of Salmonella enterica, 13 of which were non-target species. The system was evaluated in 63 samples of contaminated chicken, identifying the subspecies with specificity.

## ELISA

Currently, ELISA is the most used analytical method for tracking and sorting the presence of allergenic components in food products by industries and regulatory authorities due to its simplicity, sensitivity, and shorter response time compared to other methods [70], the method has high detection capacity, with low cost, high sensitivity and specificity [71].

According to [72]. ELISA methods can be developed based on indirect, competitive, and sandwich formats or using different types of antibodies, such as PcAbs or mAbs. Recently, considering the hazard of severe sensitization to the soybean major storage proteins glycinin and β-conglycinin, two ELISA techniques based on the sandwich and competitive formats targeted to glycinin or β-conglycinin were recently developed and extensively explored to test these proteins in model processed foods. It was concluded that the sandwich format performed better than the competitive one in anti-matrix interference and detectability.

Since the discovery of ELISA, its applications have expanded enormously due to its low cost, simplicity, high specificity, and acceptability. Despite numerous advantages, the quantitative ELISA often suffers from several drawbacks including the essential requirement of an expensive optical reader and a well-trained operator to precisely measure the color intensity. Hence, this simple and effective technique is still restricted to laboratory settings only [73].

ELISA is a routine and straightforward method for measuring aflatoxins. The possibility of a false positive in the results is high [74].



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[75] carried out a comparative study of two forms of ELISA (competitive indirect and sandwich) for the determination of soy in processed foods. The antibodies used were produced from heat-denatured glycine and reacted with the acidic subunit, which is the most thermostable fraction of glycine. The sandwich format showed greater specificity and sensitivity than the indirect competitive format. The level of detection was considered excellent, as it was able to track very low levels of soy.

In turn [76] developed a competitive ELISA tool for broad-spectrum screening of common small molecules of estrogen-damaging chemicals in foods. A dual-estrogen receptor coating was coupled onto ELISA plates and optimized bisphenol-horseradish peroxidase conjugates using dual-estrogen receptor as the competitive molecule. The method was considered to have a wide detection range and was tested on real samples (bottled water and milk), showing good detection.

## Transcriptomic analysis

The conventional approach for testing the genotoxic potential of chemicals in vitro includes a battery of bacterial and mammalian mutagenicity tests. Toxicogenomic analyses may provide information about DNA-damaging properties of test compounds but are not routinely used for the identification of a genotoxic potential [77]. Transcriptomic approaches have recently shown their value to determine transcript signatures specific to genotoxicity [78].

The transcriptome is defined as the complete set of RNA molecules in one organism. It is primarily composed of coding messenger RNA, ribosomal RNA, transfer RNA, and a variety of noncoding RNA such as small RNAs. In this context, mRNAs are of great interest in biological studies as they represent the link between genotype and phenotype, so they are essential for understanding the functional elements of the genome and the molecular constituents of cells [79].

From a biological point of view, transcriptomic analysis can reveal the current state of gene expression and provide the mechanism for transcriptional and post-transcriptional regulation [80]. The immense amount of biological data generated in the last decade by large-scale transcriptomic studies deposited in public biological databases allows secondary studies to be carried out generating viable products that can be used in the molecular diagnosis of diseases and identification of components harmful to food safety [81].

The effects of the withering process on the formation of tea flavors were investigated using transcriptome and metabolite profiling in withered tea leaves. The authors identified 3,268; 23,282 and 25,185 genes differentially expressed in the wilting process that is involved in the biosynthesis of flavonoids [82].

In turn [83] studied a transcriptome and metabolome analysis of green and red cabbage seedlings to obtain more accurate information on metabolite constituents. The kale transcriptome datasets revealed 37,149 annotated genes and several secondary metabolite biosynthetic genes [84]. Also used transcriptomic and metabolomic analysis to identify differentially accumulated metabolites of the Canarium album fruit. The analysis identified 87 metabolites, including 17 types of flavonoids.

[85] evaluated the effects of Cadmium on immune function, oxidative defense, and glycometabolism of the common carp spleen by means of transcriptome analysis. The analysis obtained 3,794 differentially expressed genes and the lives and gene functions of immune defense, oxidative, and glycometabolism were obtained and identified. The results indicated that exposure to Cadmium led to immunosuppression, oxidative stress, and impaired glycometabolism in common carp spleens.

The efficacy of linalool against Listeria monocytogenes was revealed by comparative transcriptome analysis, showing strong activity in the planktonic stage. Transcriptome analysis has demonstrated many potential mechanisms of action for linaol [86].

[87] analyzed the transcriptome of peduncles of two cashew genotypes with different firmness and color at two stages of development using the technique of RNA sequencing. The results showed that the anthocyanin content of the cashew peduncle decreased during the development of both genotypes.

The microarray technology, or DNA microarrays, makes it possible to simultaneously assess the expression of thousands of genes in different tissues in each organism, at different stages of development or environmental conditions. Microarrays are widely used in functional genomics experiments with several animal and plant species and have been gradually incorporated into different areas of zootechnical research, such as growth and metabolism, immune response to diseases, reproduction, and response to non-infectious stress factors (restriction food, exposure to toxic elements and other unfavorable environmental conditions), as well as an animal genetic improvement [88]. Other authors define it as a collection of short oligonucleotide probes that are attached to a solid surface that is specific for thousands of genes. It is based on nanofabrication and complementary probe hybridization, where there is a relative abundance of thousands of transcripts from two or more samples that can be measured at the same time.

Microarrays normally show a high background noise due to cross-hybridization, making it difficult to detect low-copy transcripts. This can also occur in combination with signal saturation, after which the range of detection is only a few hundred-fold.

Report a series of studies that used the DNA microarray technique to provide a wide range of opportunities in the identification of the target gene or sequence and a path for the studies of foodborne pathogenic microorganisms.

Developed an in situ synthesized gene chip for the detection of pathogens in foods with target products being fresh fruits and vegetables through the DNA microarray technique. Target



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genes were identified and screened by comparing the specific sequences of *Salmonella Typhimurium*, *Vibrio parahemolyticus*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli O157:H7* from the National Center for Biotechnology Information database.

## Conclusion

The use of molecular tools for the detection, identification, and quantification of bacteria, viruses, filamentous fungi, and yeasts has been widely studied, mainly for pathogenic agents in food. The main objective is the detection of species and subspecies present in food samples. The main detection techniques involve PCR and its variations.

The aid of molecular tools in the identification of potentially allergenic substances and toxins, as well as the use in the control of the authenticity of foods, to avoid fraud, and in the identification of genetically modified organisms has been promising.

The methods presented in this review proved to be fast, and efficient, with a high level of sensitivity and specificity in the identification of target analytes, as well as similar analytes. In this way, biomolecular tools can permanently replace physical-chemical, chemical, and biochemical methods, as they produce results with greater precision, reliability, and agility.

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