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Revolutionizing food integrity: Emerging methods and technologies in food authentication

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Abstract

Food authentication is now an essential process in today's food systems due to food theft and the complexity of global supply chains. This essay explores the significance of food authenticity and emphasizes how it protects consumer health, thwarts financial fraud, and maintains industry integrity. The use of traditional techniques such as eye inspection and chemical analysis to combat complex food fraud has not worked. New methods are consequently gaining recognition. These include DNA-based methods, spectroscopy, block chain traceability platforms, and sensor technology. Improvements in DNA-based authentication techniques, including polymerase chain reaction (PCR), Next-Generation Sequencing (NGS), and DNA barcoding, are revolutionizing the identification of species and preventing adulteration. These methods accurately identify mislabeled food products and confirm the authenticity of species, ensuring food labeling accuracy and lowering food fraud. Spectroscopy offers non-destructive techniques for determining the chemical makeup of food and locating adulterants. These techniques include infrared and Raman spectroscopy. Sensor technologies such as electronic tongues and noses enhance the ability to monitor food processing in real time by detecting contaminants and volatile compounds. Food traceability can be ensured, openness in supply chains can be given, and fraudulent activity can be prevented via block chain technology. The combination of block chain technology with IoT devices facilitates real-time data collecting, which enhances the efficacy of tracking agricultural goods from farm to table. Despite these advancements, there are still problems that need to be fixed, like the high expenses associated with implementing new technology and the necessity of standardizing procedures across the whole business. Case studies demonstrate how these methods can be applied in practice to stop food fraud and enhance food safety in a range of sectors, such as processed foods, meat, and seafood. Future advancements in DNA-based techniques, block chain traceability systems, and sensor technology will be essential to ensuring food authenticity. These advancements will boost consumer confidence and boost the precision of food verification, resulting in a more transparent and safe global food supply chain.

Keywords: Food authentication, food integrity, food fraud

1. Introduction

Food authentication is the process of confirming that the food matches its label (Daneis *et al.*, 2016). The techniques used to authenticate food items and ensure quality are of tremendous relevance from both legal and commercial perspectives (Drivelos *et al.*, 2012) [2]. Authenticity has always been a top priority for manufacturers, buyers, and authorities (Georgiou *et al.*, 2015) [3]. Modern technology, information and communication technologies, and breakthroughs in the basic sciences allow for the precise measurement and explanation of food origins (Cubero-Leon *et al.*, 2014) [4]. Food product authentication contains procedures that can ensure the product meets the label declarations in order to confirm the provision of applicable rules and regulations (Imran *et al.*, 2016). For the food sector, being able to trace and authenticate food goods is critical for both safety and financial reasons (Cubero-Leon *et al.*, 2014) [4]. Consumers find it crucial to identify the origin of products for health, religious, and financial reasons, as well as to prevent financial loss due to fraudulent substitution (Asensio *et al.*, 2008) [5]. Food authenticity relies on measuring characteristics that set foods apart from various sources (Danezis *et al.*, 2016) [1, 6, 42, 96].

One food fraud that has affected people's health is the inclusion of melamine to milk powders (Spink *et al.*, 2019)^[7]. For the past 20 years, the majority of food authentication techniques have focused mostly on analytical indicators that are based on either recognized substances or unidentifiable traits. (Oliveira *et al.*, 2019)^[8] (McGrath *et al.*, 2018)^[9].

2. The importance of food authentication

In the global food supply chain, food authenticity is crucial to guaranteeing food safety, quality, and integrity. It involves confirming that food goods are as described on the labels and aren't tampered with, replaced, or misrepresented in any way. This procedure is essential for safeguarding consumer health, stopping financial fraud, upholding the integrity of the sector, and guaranteeing regulatory compliance.

Protecting consumer health

Food authentication is primarily used to protect consumer health by verifying that food products are legitimate and safe to eat. Foods that have been tampered with or misrepresented can be extremely dangerous to your health, especially if you have dietary restrictions or allergies. Unreported allergies or dangerous food additives, for instance, might cause severe allergic reactions or foodborne diseases, which can have fatal or life-threatening implications.

(Zhou *et al.*, 2021)^[22]. Research has demonstrated that food fraud can also introduce unanticipated allergies or toxins into the food supply. Examples of this include diluting fruit juices with less expensive syrups or switching out one species of fish for another. (Moore *et al.*, 2012)^[18]. Authentication of food also aids in the detection of pollutants that might not be listed on the product label. For example, a study discovered that some herbal supplements were contaminated with heavy metals and prescription medications, putting customers' health at serious danger (Villena *et al.*, 2018)^[21]. Food authenticity is essential to maintaining public health since it guarantees that food products are correctly labeled and devoid of adulterants.

Preventing economic fraud

In the food sector, economic fraud is the intentional adulteration or misrepresentation of food items in order to profit financially. This kind of fraud harms the reputation of the food sector as a whole in addition to misleading consumers and causing serious financial losses for legitimate enterprises. Selling farmed salmon as wild-caught, diluting olive oil with less expensive oils, or labeling conventional products as organic are a few examples of economic fraud (Everstine *et al.*, 2013)^[14].

Food authentication, which offers scientific proof that can confirm the authenticity of food goods, is crucial in stopping such fraudulent actions. Food fraud detection and product authenticity are being verified more and more through the use of technologies such as DNA barcoding, stable isotope analysis, and spectroscopic techniques (Elliott, 2014)^[13]. For instance, species substitution a prevalent type of financial fraud in the seafood industry can be less common when fish species are precisely identified via DNA barcoding (Di Pinto *et al.*, 2015)^[12].

Maintaining industry integrity

Building and maintaining consumer trust in the food sector depends on maintaining its integrity. Food fraud and mislabeling incidents have the potential to seriously harm food brands' reputations and undermine customer trust. One prominent example of a scandal involving horse meat that occurred in Europe in 2013 and caused a considerable drop in sales for the meat sector is the discovery of horse meat in goods that were classified as beef (Cawthorn *et al.*, 2013)^[11]. The significance of strong food authentication procedures in maintaining the integrity and trust of the food sector was highlighted by this scandal. Food authenticity not only preserves customer confidence but also promotes fair competition in the sector. Food authentication helps level the playing field for ethical manufacturers and suppliers by guaranteeing that all goods satisfy the same criteria of authenticity, preventing those who participate in fraudulent tactics from getting an unfair advantage (Spink & Moyer, 2011)^[20].

Ensuring regulatory compliance

In order to guarantee adherence to laws and guidelines pertaining to food safety, food authenticity is also essential. Strict regulations have been put in place by governments and regulatory agencies worldwide to guard against food fraud and guarantee food safety. For instance, strict guidelines for food traceability and authenticity have been established by the Food Fraud Network of the European Union and the Food Safety Modernization Act (FSMA) of the Food and Drug Administration in the United States (Manning & Soon, 2014)^[17]. Food suppliers and producers can abide by these laws, avoiding fines and safeguarding the public's health, by putting strong food authentication procedures in place. Furthermore, technology for food verification including mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy have become indispensable for adhering to regulations. These techniques offer accurate information that can confirm the provenance, validity, and composition of food products, assisting regulatory agencies in efficiently enforcing food laws and standards (Rodríguez-Lázaro *et al.*, 2017)^[19].

Technological advancements in food authentication

Technological developments have greatly improved the accuracy and efficiency of food product authentication. For instance, DNA barcoding has been widely used to identify species and has made it possible to find mislabeled or replaced animal and plant items (Elliott, 2014)^[13]. Stable isotope analysis is also used to confirm the provenance of food items, which is important in detecting misleading claims about the origins of products like mislabeled wines or olive oils (Kelly *et al.*, 2015)^[15]. Spectroscopic techniques, such as Fourier-transform infrared (FTIR) and near-infrared (NIR) spectroscopy, are also being utilized more frequently in food authentication. Without modifying the sample, these non-destructive methods can examine the chemical makeup of food products to identify adulterants and confirm authenticity. (Boggia *et al.*, 2013)^[10]. As these technologies develop further, they will offer even more potent instruments for guaranteeing the safety and authenticity of food.

Advances in dna-based authentication

Food fraud prevention, food safety assurance, and authenticity verification of food products have all grown dependent on DNA-based authentication techniques. These methods make use of the distinctive genetic makeup of several species to precisely identify and validate food products. The most well-known DNA-based authentication techniques are DNA barcoding, Next-Generation Sequencing (NGS), and Polymerase Chain Reaction (PCR).

Polymerase chain reaction (PCR)

A popular molecular biology method called polymerase chain reaction (PCR) amplifies particular DNA sequences, enabling the detection of even minute amounts of DNA in a sample. Because of its extreme sensitivity and specificity, this method is very helpful for identifying species in complicated food matrices, finding pollutants, and confirming the existence of genetically modified organisms (GMOs) (Mullis & Faloona, 1987) [30].

PCR has several deviations that enhance its efficacy for food authentication:

1. Conventional PCR

This is the initial technique that makes it possible to amplify particular DNA sequences. It frequently targets distinct genetic markers to identify species in food goods.

2. Quantitative PCR (qPCR)

This method, which is also referred to as real-time PCR, measures the quantity of DNA present in a sample, which makes it perfect for identifying minute amounts of contamination or adulteration. For instance, qPCR can be used to confirm that "free-from" items satisfy safety requirements for those with sensitivity by confirming the absence of allergens like gluten or peanuts (Bustin *et al.*, 2009) [23].

3. Digital PCR (dPCR)

Through the division of a DNA sample into thousands of separate reactions, this more recent iteration of PCR provides greater sensitivity and precision than qPCR. It works particularly well to find low-level contamination or adulteration, including the introduction of unwanted species into processed beef products (Hindson *et al.*, 2011) [26].

3. Next-generation sequencing (NGS)

With the use of the potent technology known as Next-Generation Sequencing (NGS), millions of DNA fragments may be sequenced in simultaneously, giving a thorough picture of the genetic material contained in a sample. The simultaneous identification of many species made possible by this technique has changed food authentication. This is especially helpful when studying complicated food matrices like mixed meats, seafood, and processed foods.

(Mardis, 2008) [28]. NGS can be used to identify food fraud and validate statements regarding the provenance and legitimacy of food items. For instance, it can detect pollutants, confirm the existence of particular substances, and identify species in products marketed as single-species items. Using distinct DNA markers, NGS has proven to be an efficient method of verifying the identity of species and

the geographical provenance of high-value commodities, such honey and caviar (Dooley *et al.*, 2015) [24].

Utilizing next-generation sequencing (NGS) has shown beneficial in detecting hidden components in food items, supporting regulatory bodies in enforcing labeling regulations, and shielding customers from potentially dangerous or allergic substances. It is anticipated that NGS will become even more essential to food safety and authentication as sequencing prices continue to drop and computational techniques advance (Metzker, 2010) [29].

4. DNA Barcoding

A small, standardized DNA area can be used as the basis for a species' identification through the use of DNA barcoding. When physical distinctions are negligible or food products are heavily processed, making standard identification methods difficult, this method is quite successful for identifying the species (Hebert *et al.*, 2003) [25].

A particular gene region usually the ribulose-1,5-bisphosphate carboxylase (rbcL) gene for plants or the cytochrome c oxidase I (COI) gene for animals is sequenced and compared against a reference database of known sequences in DNA barcoding. If a match is discovered, the species can be recognized with certainty. This method has been effectively used to verify the authenticity of many different food items, such as meat, fish, and botanical substances (Kochzius *et al.*, 2010) [27].

DNA barcoding, for instance, has been used to detect seafood that has been incorrectly classified, such as when inferior fish species are marketed as superior types. DNA barcoding contributes to the preservation of consumer rights, the prevention of economic fraud, and the integrity of the food supply chain by offering a dependable and consistent approach for species identification (Wong & Hanner, 2008) [31, 172].

Emerging food authentication methodologies to ensure safety and quality of food

Food authenticity is essential to ensuring the integrity, safety, and quality of food items worldwide across the global food supply chain. As agriculture and distribution networks become more complicated, there is an increasing demand for effective methods to verify the authenticity of food. Food authentication uses a variety of techniques and instruments to detect food fraud, ensure label accuracy, and protect consumers from potential health risks (Lolli & Caligiani, 2024) [32].

The rise in food fraud, which includes adulteration, mislabeling, and counterfeiting, has required the development and application of advanced verification methods.

One of the primary motivations for food authentication is safety for public protection. Food products that are tainted or mislabeled can pose a serious risk to one's health, particularly for people with allergies or dietary sensitivity (Brooks *et al.*, 2021) [33].

For instance, eating food containing undeclared allergens may cause severe allergic reactions, which are occasionally even fatal. Food fraud also costs customers and legitimate manufacturers a lot of money in addition to eroding consumer confidence. Food fraud has a huge financial

impact; estimates put the annual losses to the global food industry from fraud in the billions (Smyth *et al.*, 2021) ^[34].

Therefore, preserving the legitimacy of the food supply chain is essential for the economy and consumer protection.

The complexity of today's food supply chains, which usually involve multiple middlemen and cross-border transactions, makes it very challenging to ensure food authenticity. Traditional methods, such as visual inspection and basic chemical analysis, are no longer sufficient for spotting sophisticated fraud (Prandi *et al.*, 2022) ^[35].

The globalization of the food sector has increased the possibility of food fraud and made it more difficult to confirm the authenticity of food products. As a result, there has been a noticeable increase in the development and application of state-of-the-art technology meant to improve the accuracy and consistency of food authentication processes. (Negi *et al.*, 2021) ^[36].

Since these technologies may offer detailed information on the components, provenance, and manufacturing history of food products, fraud can be caught more successfully with their assistance.

Emerging food authentication approaches leverage scientific discoveries of the modern era to overcome the limitations of conventional methods (Musio *et al.*, 2020) ^[37]. These techniques include DNA-based strategies, blockchain-based traceability systems, spectroscopy and spectrometry, and sensor technologies. Since each of these technologies offers special advantages in terms of precision, speed, and adaptability, they are all highly helpful for ensuring the safety and quality of food.

DNA-based methods such as polymerase chain reaction (PCR), DNA barcoding, and next-generation sequencing (NGS) have demonstrated remarkable efficacy in determining species identity and identifying food adulteration. These methods aid in ensuring label accuracy and preventing food fraud by accurately identifying the plant and animal species included in food products. The method of identifying an organism as a member of a particular species through the use of a short genetic identifier found in its DNA is known as DNA barcoding (Liu *et al.*, 2017) ^[38]. Amplification of certain DNA sequences by PCR allows for the detection of even minuscule amounts of DNA. NGS's high-throughput sequencing capabilities make it possible to thoroughly analyze food samples.

Due to their speed and accuracy in offering a non-destructive assessment, spectroscopic and spectrometric procedures are often used in food verification. Techniques including mass spectrometry (MS), mid-infrared spectroscopy (MIR), and near-infrared spectroscopy (NIR) are used to examine the chemical composition of food products. Because these techniques may detect contaminants, adulterants, and authenticity marks, they are

essential for ensuring food safety and quality. Using NIR and MIR spectroscopy, infrared light interacts with food samples to give information about their molecular composition. Food chemical composition may be well understood thanks to mass spectrometry, which uses the ratio of mass to charge to identify compounds.

In the subject of food verification, sensor technologies such as electronic noses (e-noses) and tongues (e-tongues) have shown great potential. These sensors mimic human taste and smell receptors to identify and detect volatile compounds and flavor characteristics. They are particularly useful in figuring out whether various food items have been ruined, infected, or adulterated. The potential applications of inexpensive, mobile sensors for real-time food verification are increasing. E-noses employ a range of gas sensors to identify specific volatile chemicals, whilst e-tongues use taste sensors to ascertain the chemical composition of food samples.

Blockchain technology is revolutionizing food traceability by providing a safe and transparent way to track food products from farm to table. Blockchain verifies the authenticity and legitimacy of food supply networks by keeping a record of each transaction in a decentralized ledger. Food fraud can be prevented by using this technology to track the origin, processing, and distribution of food goods (Danezis *et al.*, 2016) ^[1, 6, 42, 96]. The immutability of blockchain records boosts trust between wholesalers, producers, and government agencies. Combining blockchain technology with the Internet of Things (IoT) can provide real-time data on food commodities throughout the whole supply chain (Kuang *et al.*, 2024) ^[39].

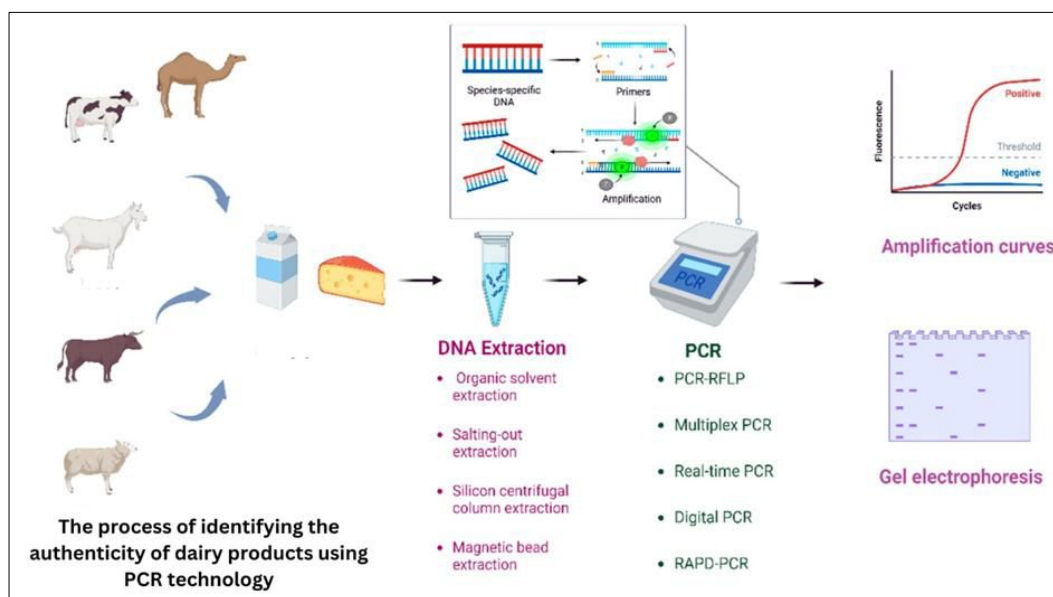
By putting these cutting-edge technologies into practice, the food industry may significantly increase its ability to authenticate food goods, guaranteeing safety, quality, and consumer trust. To prevent food fraud and maintain an open and secure food supply chain, further advancements in spectroscopy, blockchain traceability, sensor technologies, and DNA-based approaches will be crucial (Longchamps *et al.*, 2024) ^[40].

Advancement in dna-based authentication systems

Food safety and quality assurance are undergoing a revolution thanks to the development of DNA-based authentication. These cutting-edge techniques guarantee a certain caliber of the kind that guarantees food items are what they say they are and satisfy food regulations. According to Vinothkanna *et al.* (2024) ^[41], three main DNA-based techniques are Next-Generation Sequencing (NGS), DNA Barcoding, and Polymerase Chain Reaction (PCR). Every one of these approaches has special advantages and applicability to the changing landscape of food authenticity.

Polymerase chain reaction (PCR)

Overview of the technique



Definition The polymerase chain reaction (PCR) is a widely used molecular biology technique that rapidly makes millions to billions of copies of a specific DNA sample, allowing scientists to take small samples from the tissues and amplify by direct sequencing (Abidin *et al.*, 2023) [43]. The amplification also means that researchers can closely study the DNA - even from tiny or damaged traces of genetic material. PCR includes various cycles of denaturation (which is separating DNA strands), annealing (binding primers to the target sequences) and extension or synthesis of new strands, which are catalyzed by a special enzyme, known as DNA polymerase.

Utilization in food authentication

For food authentication, this technique is widely used for the detection of unique DNA sequences linked to a species or an ingredient in some product. Such capacity is important to verify foods as well, recognize any adulteration or assure - the finally production contains what its pretenses. For example, PCR can detect the presence of allergens in food products, verify that a meat product is composed entirely from certain species (e.g. horse), identify genetically modified organisms present within plant material and be used for many other applications setwheeras summarized (Kanwal & Musharraf, 2024) [46]. The high sensitivity and specificity of PCR make it a crucial technique for food safety, thus guaranteeing consumer confidence.

Next-generation sequencing (NGS)

Overview of the technique

Millions of DNA fragments can be sequenced simultaneously using Next-Generation Sequencing (NGS), a high-throughput DNA sequencing method. Unlike traditional sequencing methods, next-generation sequencing (NGS) can generate massive amounts of data quickly, which makes it possible to thoroughly examine complex genomes (Kurosaki *et al.*, 2024) [44]. After breaking apart the DNA, the pieces are amplified, adaptors are added, and they are sequenced simultaneously. The sequence is then put back together using data analysis.

Utilization in food authentication

NGS offers a wide range of applications in food verification since it can analyze complex combinations and detect several species in a single sample. This method is particularly useful for detecting food fraud, such as the substitution of pricey ingredients with less expensive ones. NGS can also be used to verify the authenticity of products advertised as having specific species, such as fish or meat, by comparing the collected DNA sequences with reference databases (Yan, 2024) [45]. Additionally, NGS can detect contaminants and pathogens, ensuring that food products follow safety guidelines. The wealth of data generated by NGS provides a comprehensive understanding of the food's genetic composition, which makes it a vital tool in modern methods of food authenticity.

DNA Barcoding

Overview of the technique

A short, standardized portion of the genome is used in the DNA barcoding method of species identification. Usually, this is a portion of the *matK* and *rbcl* genes in plants, the mitochondrial cytochrome c oxidase I (COI) gene in mammals, or the ITS region in fungi (Enitan-Folami & Swalaha, 2023) [47]. By comparing the DNA sequence of the barcode region with reference sequences kept in a database, it is possible to accurately detect the species of an organism.

Utilization in food authentication

In the food verification process, DNA barcoding is a helpful technique for verifying the species identity of both raw and processed food items. It is commonly used to identify species that have been mislabeled or substituted, particularly in the seafood industry where it is common practice to swap out pricey species for less expensive ones (Lanubile *et al.*, 2024) [48].

DNA barcoding can also be used to authenticate plant-based products, herbal supplements, and other things where species substitution may occur. The method is a vital tool for ensuring regulatory compliance, protecting consumers from food fraud, and confirming that food products are

genuine and meet label standards due to its precision and dependability (Zambianchi *et al.*, 2021) ^[49].

5. Advances in DNA based authentication

Polymerase chain reaction (PCR)

A scientific technique called polymerase chain reaction, or PCR, is used to create millions of copies of a gene of interest. (Nazir, 2020) ^[140].

PCR involves using of short pieces of synthetic DNA called primers to select a part of the gene to amplify, followed by several rounds of DNA synthesis to amplify that part. PCR is a powerful molecular biology technique because of its several benefits like speed, faster results and low cost.

Working principle of PCR is simple. As it is known that it creates millions of billions of copies of target DNA, so PCR works just like a chain reaction. It codes a specific DNA and makes its copies by using polymerase enzyme.

Three major steps in PCR technique are denaturation, annealing and extension. In first step denaturation of DNA is occurred by heating it at a temperature of 95°-97° C and thus the double stranded DNA is splitted into two strands. In the second step, primers bind to the complimentary sequence of the DNA. In this step, the temperature is lowered to about 50°-60° C. In the last step, extension of primers is done by Taq Polymerase at a temperature of 72° C. Taq Polymerase adds dNTPs (deoxynucleotides triphosphates) to elongate the primers (Joshi & J.D, 2010) ^[134]. These steps are repeated in cycles i.e. approximately 25-30 cycles to amplify the DNA. The amplified DNA thus can be studied by various methods such as gel electrophoresis, real time PCR or DNA sequencing (Jamil *et al.*, 2024) ^[136].

PCR in food safety and quality

Food products must not contain any kind of microbes or their toxins or any other substances that are harmful to human health beyond a certain limit. Different analytical methods are used to detect hazards in foods such as chromatography, spectrophotometer, PCR and different microbial methods such as streaking etc. PCR technique plays an important role to ensure the quality and safety of food in different fields (Bonilauri *et al.*, 2016) ^[138].

Detection of microbes

Microbial analysis is important to ensure the safety and quality of food (De Medici *et al.*, 2015) ^[139]. There are several techniques that help to identify the presence or absence of microorganisms in a food sample. But from different researches it is proved that PCR gives more reliable and efficient result than other simple cultural methods. The reason behind this is that other cultural methods detect only viable microbial cells (Bonilauri *et al.*, 2016) ^[138]. But there are several phases exist between live and dead bacteria such as criptobiotic (a state in which all metabolic processes stop including reproduction, development and growth), dormant, latent or lag phase, in which they do not grow or form colonies on culture media but perform other activities (Davey, 2011) ^[137].

Considering the different phases between live and died bacteria, it is observed that when the bacteria are dormant, criptobiotic or lag phase the bacterial cell don't replicate or reproduce thus can't be identified by simple cultural methods. But in PCR technique, the results are quite different. PCR detects all forms of microbes as it uses gene of interest to analyze the sample.

Following table shows the number of positive results of Salmonella and Listeria Monocytogenes by PCR and simple cultural methods of an experiment of 3 years: -

Table 1: Comparative Detection of *Salmonella* and *Listeria monocytogenes* by Cultural Methods and PCR

Evaluation	Salmonella	Listeria Monocytogenes
No. of samples analyzed	5962	4133
No. of samples positive by cultural methods	26	65
No. of samples positive by PCR	130	159
PCR/ cultural methods ratio	5	2.44

This study confirms the sensitivity of PCR as compared to cultural methods (Bonilauri *et al.*, 2016) ^[138].

Detection of genetically modified crops

Genetically modified crops are those that are produced by altering their genes through genetic engineering methods. This is done to alter or inherit some traits into the crops like disease resistance, improved nutritional value, or to increase the yield of crops. Genetic modification of crops has controversy in some aspects as some people thought that it might possess adverse effect on human health and may contribute in the occurrence of new diseases. Others say that genetically modified crops help in overcoming future global challenges of food security and provide better nutrition.

Due to this conflict, it is advised to mention GM crops on the labeling of food products. This is specifically done to make the consumers know what they are consuming. Also, GMO detection methods are implemented to ensure that labeling is done properly which is ensured by law enforcement agencies. PCR technique detects both quality and quantity of GM crops. PCR use some specific primers

to detect these genetically modified genes by amplifying them by its three basic steps denaturation, annealing and extension. This testing is basically done to ensure that food products are labeled properly if it uses genetically modified crops (Jamil *et al.*, 2024) ^[136].

Allergens detection

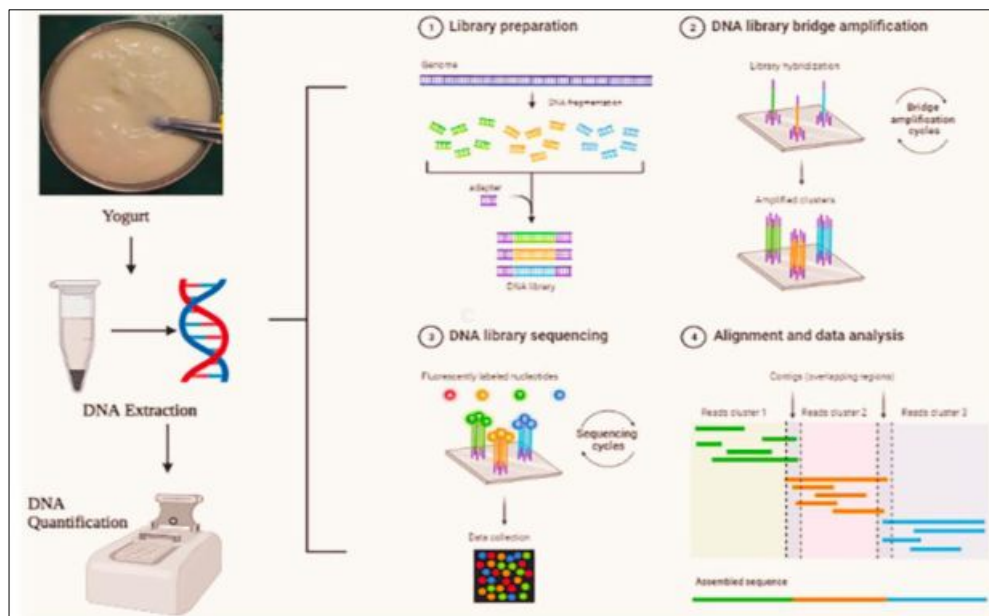
Food allergy is an adverse immune response of a person's immune system to a certain food protein which is considered harmful to the body. It is reported that most of the food allergies fall in the category of Immunoglobulin E (Ig E) as they trigger the immune system to produce Ig E antibodies. Exposure to a small amount of allergen may cause serious health problems and sometimes even death (Kopko *et al.*, 2024) ^[142].

Depending on the severity of exposure to allergens that are not mentioned on the label may pose serious health issues to the allergic person. So, allergen warning on the product label is a precautionary measure to protect the consumers who are facing this problem (Remington *et al.*, 2020) ^[143]. Traditional detection methods used to detect allergens in

food are ELISA (Enzyme Linked immunosorbent assay), chromatography technique, PCR. The ELISA technique gives the results within few hours. But there are some cons of this method such as poor sensitivity and lack of replication. PCR is more efficient and effective method for analyzing allergens in food. This tool is employed officially for analyzing allergens in Germany and Japan (Zhu *et al.*, 2024) ^[144].

But PCR is powerful detection method for the identification of allergens because of its specificity and sensitivity. PCR allows the detection and quantification of allergens for the verification of food labeling; in case the product may contain any allergen that is not mentioned on the product label. This helps to make the industries compliance with the food labeling requirements (Holzhauser & Roder, 2015) ^[141].

Next generation sequencing



Next generation sequencing (NGS) is gaining its popularity in different fields for the purpose of diagnosis, food analysis, for the investigation of outbreaks and antimicrobial resistance etc. Due to the continuous development in NGS, it is improving in the aspect of quality and cost (Jagadeesan *et al.*, 2019) ^[145]. NGS has revolutionized many fields of biology, animals and plant genome and study of gene transcription (Goodwin *et al.*, 2016) ^[146].

First of all, a DNA of good quality is essential for sequencing. Then template is prepared by using the library containing nucleic acids i.e. DNA or cDNA. These libraries are constructed by these nucleic acids and are then amplified for sequencing (Berglund *et al.*, 2011; Quail *et al.*, 2012) ^[152, 153]. To obtain nucleic acid sequence from the amplified libraries, the Ion Torrent PGM and the MiSeq both rely on sequencing by synthesis. Sequencing occurs by a continuous series of cycling and flooding of nucleotides in a sequential order. The PGM and MiSeq are two different methods to detect the sequence of nucleotides (Quail *et al.*, 2012) ^[153]. Once the sequence is completed, raw sequence data undergoes through several analysis steps. Analysis of the sequence can include a wide variety of bioinformatics assessments, including genetic variant calling for detection of SNPs or indels, detection of novel genes or regulatory elements, and assessment of transcript expression levels (Gogol-Doring & Chen 2012) ^[154].

GMO identification

The NGS technique can be used to identify the genes of GMOs (Fraiture *et al.*, 2017) ^[149]. It has been estimated that food production needs to increase by 2050 due to increase in world's population (Delaney, B. 2015) ^[150]. The adaptation of GM crops in agriculture has increased the production of

crops due to their modified traits such as disease and insects' resistance etc. (James, C. 2009) ^[151]. Next generation sequencing allows sequencing of multiple samples which are differentiable during analysis on the basis of unique barcodes added during preparation step. As compare to Sanger technique, next generation sequencing reduces the need of fragment cloning method. The millions of copies are analyzed using bioinformatics tools (Buermans *et al.*, 2014; Escalona *et al.*, 2016) ^[157, 158]. NGS uses two main strategies for detection of GMOs. Firstly, NGS whole-genome sequencing (WGS) uses the sequence of whole DNA extracted from a sample without any a prior knowledge of the nucleotide composition. Secondly, NGS targets the sequence of interest, isolated earlier from the whole DNA extracted from a sample. This approach is considered to be a targeted because selecting the sequence of interest requires a minimum of knowledge (Yang *et al.*, 2013; Buermans *et al.*, 2014; Kovalic *et al.*, 2012; Wahler *et al.*, 2013; Young *et al.*, 2015) ^[157, 159, 160, 161, 162].

Identification of microbes

Culturing method is a traditional way for the identification of microbes that depends on the isolation and culturing of microbes on different culture media (Amann *et al.*, 1995) ^[164]. The selective isolation of microbes requires different growth factors or conditions that are present in natural habitat and thus can't be provided in laboratory conditions. Other factors include low or high pH of food, reduced water activity (A_w) or other conditions that may leave microbes in physiological viable but not in cultivable state. Moreover, microbes that are high in number may mask the identity of those that are low in number and so can't be identified in culture media. Due to such limitations, traditional culturing

methods sometimes even fail to detect such microbes (Ercolini *et al.*, 2001) ^[163].

As compared to culturing methods, the number of DNA sequences analyzed by next generation sequencing (NGS) is comparatively higher and the results are more accurate. This technology can be used in two different ways i.e. shotgun sequencing (sequencing the total microbial nucleic acid) and targeted sequencing (gene specific sequencing) (Wilmes *et al.*, 2008; Solieri *et al.*, 2013) ^[165, 166]

DNA Barcoding

DNA barcoding is a method for the identification of eukaryotic organisms that is based on the analysis of small, standardized gene sequence (Baracaccia *et al.*, 2015) ^[175]. DNA barcoding amplifies short DNA fragments of mitochondria in case of animals and chloroplasts in case of plants (Baracaccia *et al.*, 2015) ^[175]. Due to increase of awareness about food in consumers through social media and advertisement, people pay more attention about the safety and quality of food (Barbuto *et al.*, 2010) ^[168].

DNA barcoding is an effective method to analyze the origin of raw material, to ensure the quality and also for the testing of adulteration (Galimberti *et al.*, 2013) ^[148]. DNA barcoding can be applied to both fresh and processed food products for specie identification whether the products are made from single or mixed specie (Baracaccia *et al.*, 2015) ^[175].

Food traceability

With the continuous development in the production of different food products, the risk of food contamination and adulteration is also increasing that may pose serious health issues to the consumers. DNA barcoding tool can be used to ensure the authenticity and traceability of food (Dawan & Ahn., 2022) ^[170].

DNA barcoding shows effective results in the analysis of fruit based processed products like juices, jams etc. The use of specific primers to detect the presence of adulterants in plant-based products help to ensure the safety and quality of food (Faria *et al.*, 2013) ^[173]. This method can also be used for the detection of allergens in foods. Hazelnut is a very common allergen as a very small amount of it can pose serious health problems. So, the use of hazelnut or its contamination could cause risk to the consumer's health.

Taking into account the health of consumers, regulatory bodies have made the regulations of printing allergen warning on the label to make the consumers know if the material they are allergic to is present in the product or not (Madesis *et al.*, 2013) ^[174].

A well-established traceability system is important that can be used for traceability in animal-based products. DNA barcoding is effective tool for this purpose as whole carcass is rarely displaced in the market as compared to their processed products like ready to cook and ready to eat products. So, as the market of processed products is increasing in the market, adulteration and food fraud is also increasing. To overcome this problem, DNA barcoding is an essential tool to ensure the proper labeling of food products and also ensure product quality (Baracaccia *et al.*, 2015) ^[175].

Seafood traceability

Seafood possesses a wide range of eukaryotic vertebrates. DNA barcoding helps to identify the fish species from whole fish, fillets, fins or any other type of fish that is preserved. DNA barcoding can also be applied successfully to cooked or processed fish. Samples that are degraded during processing methods require the shorter fragments which are called mini barcodes (Becker *et al.*, 2011) ^[167]. Even though consumers are gaining knowledge about the quality and safety of food, but their inability to differentiate between different species of fish lead to mislabeling on the food package (Marko *et al.*, 2004) ^[171].

DNA barcoding has been successfully used to identify the different species of fish even though it has undergone any processing technique like grilling, barbecuing etc. or any other industrial processing like heat exposure, pressure etc. (Barbuto *et al.*, 2010; Wong and Hanner, 2008) ^[31, 172, 168].

The Fish Barcode of Life Initiative (FISH- BOL) is an aid to assembly the standardize sequence of all fish species. The primary goal of FISH-BOL is to gather the DNA sequence of all species of fish in the world, approximately 31,000. The advantages of fishing barcoding include specie identification, highlighting specimen and enable identification where traditional methods cannot be applied. The following table shows the progression of fish barcoding in different regions by FISH-BOL:

Table 2: Regional Progress in Species Barcoding

Regions	Species	Specie barcoded	Progress
Southeast Asia	12,140	2019	16.6%
Europe	2028	391	19.3%
Australia	8623	2449	28.4%
Africa	8980	1216	13.5%
North America	8112	2239	27.6%

(Becker *et al.*, 2011) ^[167]

6. Applications of spectroscopy in food authentication

6.1 Terahertz spectroscopy

Terahertz (THz) spectroscopy is becoming more and more popular in the food technology and processing industry as a useful technique for food authentication. It evaluates matter's characteristics in the presence of an electromagnetic field. The term "terahertz" describes the range of electromagnetic radiation that falls between the microwave and far-infrared (IR) regions. It is represented by a frequency that falls between 0.1 and 10 THz, or 3000 and 30

µm in wavelengths (Zouaghi W *et al.*, 2013) ^[50]. Multiple terms, including T-waves, terahertz gap, terahertz light, terahertz waves, T-lux, or T-light, can be used to refer to THz. By comparison, conventional spectroscopic and imaging techniques are very well-established and frequently employed, while THz wave investigation and application are still in their infancy. THz spectroscopy comes in three essences: time-resolved THz spectroscopy (TRTS), THz emission spectroscopy (TES), and THz time-domain spectroscopy (THz-TDS) (Kiwa T, *et al.*, 2003) ^[51]

Because of the recent developments in THz sources and detectors, this technology has shown significant promise as a powerful and innovative non-destructive instrument for real-time monitoring and quality control of food processes (Corsi C & Sizov F.,2014) ^[52]. THz waves are useful because they contain photon energies between 1 and 100 meV, which are within the critical energy range for a range of materials and biomolecules. They can also pass through materials that are opaque to visible light. Many chemical compounds and microbiological elements have distinct spectra at THz frequencies that can be utilized to identify and quantify these threats.

The broad frequency range of THz waves allows for the detection of molecular interactions in food components brought about by hydration, van der Waals force, and hydrogen bond formation. These phenomena include biological macromolecules like DNA and proteins' phonon, as well as vibrational and rotational energy levels (Ajito K, *et al.*,2017, Chen Z *et al.*,2015) ^[53, 54]

In the food sector, THz spectroscopy is used to find dangerous compounds, microorganisms, antibiotics, additives, and toxins. Compared to established, conventional identification methods, THz spectroscopy technology may effectively discriminate between various contaminated dairy products based on their fatty acid composition, avoiding the need for sample pretreatment (Liu J.,2016) ^[55].

The K-value index is used in the meat business to assess pig freshness, which is a critical quality factor. Eighty pork samples were exposed to K-value predictions with a 7.6 GHz frequency resolution using attenuated total reflectance mode THz spectroscopy, all while maintaining the pig's original structure. Prediction models were created using multivariate statistical approaches including the BP-non-linear ANN method and the PCR linear regression methods. The results demonstrated how well THz spectroscopy, in conjunction with a prediction system, can evaluate the freshness of pork. Moreover, the THz spectrum of fresh pork was found to lie between 0.2 and 2.2 THz (Liang, QI *et al.*,2019) ^[56]. The non-destructive identification of dangerous compounds, food pathogens, and hazardous components has proven to be effective with THz technology. Numerous studies have demonstrated that when THz is combined with chemometric techniques, hazardous and deadly compounds in food samples can be quickly identified and assessed in even less than five seconds. Although THz spectroscopy has made strides recently in the quantification of hazardous substances, the technique's detection limit still has to be increased. It occasionally misidentifies hazardous compounds in neutral samples, even when they are absent from the samples. Time-dependent THz pulse spectrum analysis could help prevent this problem (Trofimov VA & Varentsova SA.,2016) ^[57]. This method is popular because it is an effective instrument with excellent detection and quantification capabilities.

6.2 Laser induced breakdown spectroscopy

The food analytical tool known as laser stimulated breakdown spectroscopy (LIBS) was established and recommended as soon as the laser was discovered in 1960. The basic principle of LIBS is that a food sample interacts with a strong laser beam, which causes the dielectric to break down and produces heated plasma in the process. Plasma is made up of atoms, molecules, electrons, and ions that have energies higher than that of their ground states. A

spectrometer that provides identification and quantitative statistics of each element present in the sample detects the unique radiations that these molecular species produce when they return to their ground state (Markiewicz-Keszycska M *et al* 2017) ^[58] A spectrum covering the wavelength range of 190 to 850 nm is often analyzed using LIBS.

Initially LIBS was utilized for industrial (Noll R *et al* 2018) ^[59], space (Cremers DA *et al.*,2014) ^[60], cultural heritage (Anglos D *et al.*,2014) ^[61], and environmental (Zhang Y *et al.*,2021 ^[62], Chen T *et al.*,2020) ^[63] applications. However, the scientific community has recently become increasingly interested in the incorporation of LIBS for food verification. It can be used to determine the geographic origin of food as well as to detect adulterants and heavy metals in food products. (Katerinopoulou K, *et al.*,2020, Baskali-Bouregaa N *et al.*,2019) ^[64, 65].

A LIBS technique was established to illustrate milk quickly and easily. Convolutional neural networks (CNN) and conventional machine learning techniques were used to accurately and consistently identify a variety of contaminated milk powders. Out of the four machine learning algorithms support vector machines, random forest (LDA), linear discriminant analysis, and k-nearest neighbor the combination of LIBS and CNN was found to be a simple, trustworthy, and genuine method for identifying adulterants.

(Huang W *et al.*,2022) ^[66].

In a further experiment, the element compositional discrepancies between meat species were employed by LIBS to identify them. Meat from several species, including pig, cattle, and chicken, was mixed together, palletized, and subjected to the LIBS treatment during the experiment. The principal wavelength of the laser utilized to ablate the samples was 1064 nm. Q switched mode with gate delay of 300 ns, integration duration of 1.05 ms, pulse width and rate of repetition of 4 Hz. The energy used in each pulse was 38 mJ. Both statistical and mathematical techniques were used to examine the collected spectra. The limit of detection (LOD) and R² (coefficient of determination) values for contaminated beef including chicken and pork were calculated to be 0.999, 2.0, and 0.994, 4.4%, respectively (Bilge G *et al.*,2016) ^[67]. If this approach could detect minute and trace minerals in complex organic matrix at extremely low concentrations, its use for authentication reasons would increase. From the foregoing explanation, it can be concluded that LIBS can offer quasi-non-destructive in-situ chemical analysis, which can be used for food authenticity in a variety of contexts and without sample preparation.

6.3 Hyperspectral imaging

The combination of spectroscopic approach and imaging technology has led to the development of hyperspectral imaging technique, a non-invasive method for identifying and detecting food quality that expresses massive amounts of spectral and spatial data for several samples at once (Huang H *et al.*,2014) ^[68]. This imaging method allows for the analysis of a sample's morphological and compositional characteristics by taking pictures from various spatial orientations (Liu D *et al.*,2015) ^[69]

Compared to other spectroscopic techniques, such as NIR, which only provides spectral information about a single spectrum, hyperspectral imaging (HSI) has the advantage of being able to draw complete spatial information. However,

spatial information is lacking in other spectroscopic methods. By merging spectral and spatial data, HIS offers a visual depiction of the elements in a sample that will be assessed. (Feng Y-Z *et al.*, 2013) ^[70].

Goetz, Vane used this technology for the first time in the 1980s by combining spectroscopy and digital imagery. These days, the food, pharmaceutical, and agricultural industries all make extensive use of this technique. The fundamental units of hyperspectral photos are hypercubes, which are 3-D data cubes made up of many sequential spectral bands for each spatial point in a sample (Edwards K, *et al.*, 2021) ^[71, 81].

There is an identical spectrum for every pixel that is visible in an image. Every spectrum has the ability to work similarly to a biochemical fingerprint or signature, making it possible to identify and define the chemical components of a particular sample (Williams PJ *et al.*, 2013) ^[72]. HIS can examine how different chemical components are distributed within a sample, in contrast to NIR spectroscopy. This enables the analysis of mixed samples and the determination of the constituent chemical components of the sample (Burger J & Gowen A *et al.*, 2011) ^[73]. A computer system is connected to a near-infrared-hyperspectral imaging system that includes a camera for taking pictures, a spectra analyzer, a detector, an illuminator, and a translator (Williams PJ, 2009) ^[74].

Three imaging modes or procedures are available for creating a hyperspectral image of a sample from various angles: look down imaging, also known as wavelength scanner, push-broom (movement) imaging, and whiskbroom (diffusion of substances) imaging, also known as point scanner (Gowen AA *et al.*, 2007) ^[75, 79]. Hypercube retrieval is made possible by the whiskbroom configuration, which rotates the sample or the detectors in multiple spatial directions while scanning every point on the sample. This technique is perfect for microstructural scanning because it produces images with outstanding spatial resolution (Qin J *et al.*, 2013) ^[76]. With the push-broom setup, an image can be acquired in under 20 seconds, depending on the field and amplification (Ortega S *et al.*, 2019) ^[77]. For at-line or online conveyor belt applications, a 2-D detector is appropriate because it allows for the line-by-line collection and recording of spatial and spectral data (Kamruzzaman Metal., 2013) ^[78].

An image of the whole field of view is captured when the spectrophotometer and sample are at rest in the stare-down position. The HSI approach gathers a sequence of images by working with one wavelength range at a time. The final 3D image is created by combining recorded images with a large collection of photos called a hypercube (Gowen AA *et al.*, 2007) ^[75, 79].

All biological materials normally reflect, scatter, absorb, or emit electromagnetic radiation. Different materials interact with light differently for each wavelength. This foundation supports HSI. This effect is caused by differences in their basic structure and chemical composition. A fingerprint or spectrum signature is a recognizable pattern that is specific to a given material and is produced when the material interacts with light. Thus, by finding, defining, and separating them, the usual spectral signature acquired is used to discriminate across sample kinds and classes (Elmasry G *et al.*, 2012) ^[80]. Because of the surface heterogeneities, scattering frequently causes interferences in spectroscopic data (Williams). Hence, spectral pre-

processing is typically recommended to eliminate certain non-chemical deviations or biasness from the collected spectra prior to getting the most pertinent analytical information. The most important information is then extracted from the data matrix and any potential differences between the samples are highlighted by subjecting the data to sophisticated chemometric algorithms (Edwards K *et al.*, 2021) ^[71, 81].

As a result, when combined with the proper chemometric models, this technology has made it possible to fully classify, characterize, and detect adulterants as well as provide the best authentication strategy for a variety of food products, such as milk, yogurt, and eggs. According to Kamruzzaman, Makino adulteration with horsemeat in comminuted beef samples can be detected utilizing a mix of visible near-infrared and hyperspectral imaging technologies (Kamruzzaman M *et al.*, 2015) ^[82]. Four wavelengths -515, 595, 650, and 880 nm were selected for the investigation using the PLSR model. With a R² value of 0.98, the degree of adulteration in horsemeat could be accurately predicted. The researchers found that by combining VIS-NIR spectroscopies with HSI technology, adulteration in minced beef may be promptly and nondestructively identified.

In a similar vein, Zheng, Li (Zheng X *et al.*, 2019) ^[83] used VIS-NIR and hyperspectral imaging to detect counterfeit duck flesh in minced lamb meat samples. The PLSR model yielded a successful model performance with an R² value of 0.99, and fourteen wavelengths were chosen. Pork contamination in comminuted beef can be detected using hyperspectral imaging. Additionally, according to research, this technique can successfully differentiate samples of pure beef and pork from those that have been tampered with (Ropodi A *et al.*, 2015) ^[84]. According to Shafiee S *et al.* (2016) ^[85], HIS can also be used to detect forgeries in honey samples and validate the genuineness of bee-derived pure honey.

6.4 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy has shown to be a successful method for food authenticity verification along the food chain, from raw materials to final goods, for quality control and the monitoring of fake labeling. NMR spectroscopy is a quantitative technique where the number of nuclei produced under given conditions is exactly proportional to the area of the NMR signal. Numerous nuclei can be used for sample analysis for identification and categorization (Otte DA *et al.*, 2014) ^[46].

Originally, the only uses of NMR in the food industry were for low-resolution moisture analyses. Nevertheless, with the advancement of technology, it is now possible to conduct high-resolution investigations of both solid and liquid samples for authentication, classification, and structural and compositional characterization (Spyros A & Dais P., 2012) ^[87] and thus facilitates the molecular interactions between food ingredients.

(Fernandes A *et al.*, 2015) ^[88]. Because it doesn't require sample preparation and has great precision, improved consistency, and is non-destructive, NMR analysis is a valuable tool for assessing diverse food systems (Jakes W *et al.*, 2015) ^[89].

Research demonstrates how effectively NMR spectroscopy may be used to distinguish and identify beef from horse meat. Utilizing low field NMR to analyze the lipid components of horse and beef samples, sufficient

differences were found to enable the development of classification models. After examining the research studies, it is possible to draw the conclusion that NMR has become a viable method for verifying the authenticity of meals derived from animals.

6.5 Raman spectroscopy

A non-destructive spectroscopic method called Raman spectroscopy creates shifted energy frequencies by light scattering. The inelastic scattering of light from the samples to be studied produces Raman signals, and the resulting frequency shift reveals the stretching vibrations involved (Craig AP *et al.*, 2013) ^[90].

By creating a fingerprint of a representative molecule from the resultant spectra, one may compare molecules in various samples and carry out structural and qualitative characterizations (Yang D *et al.*, 2011) ^[91].

Using NIR, Raman, and most importantly, MIR spectroscopy, methods for authenticating and detecting adulteration in several food categories have been created with ease, convenience, and speed. In order to detect adulteration in comminuted meat products including beef offals, Zhao, Downey (Zhao M *et al.*, 2011) ^[92] used dispersive Raman spectroscopy in conjunction with multivariable data analysis. By comparing the amounts of adulteration in original and offal-adulterated beef burgers, PLS-DA was utilized to analyze the spectral data for the purpose of verifying the authenticity of samples of beef burgers manufactured in accordance with market formulae. PLS-DA models identified tainted and authentic samples with high accuracy. Additionally, the quantity of excess fat and total offal in the samples could be measured thanks to PLS regression models.

Li, Shan (Li, Shan *et al.*, 2012) ^[93] tried to discover adulterants such additional sweeteners, such as high fructose corn and maltose syrup, in natural honey by using Raman spectroscopy in conjunction with chemometrics. When used in conjunction with PLS-LDA, Raman spectroscopy was able to accurately identify samples contaminated with high fructose corn syrup with an accuracy of 91.1% when compared to authentic honey samples, and 97.8% when it came to samples contaminated with maltose syrup when compared to pure honey samples.

Nonetheless, the PLS-LDA chemometric technique produced an overall accuracy of 84.4% in the classification of honey adulterants, whether they were maltose syrup or high fructose corn syrup. The Raman spectrum is an innovative method for confirming the authenticity and checking for adulteration in milk since water appears to have very little effect on it, making it a direct measure of aqueous solutions with an easy sample preparation process. Different varieties of milk powder were identified by their chemical fingerprints through the application of Raman spectroscopy and chemometrics in order to distinguish between samples (Karacaglar NNY *et al.*, 2012) ^[94].

Melamine levels in milk powder were measured online using a handheld Raman spectrometer. In order to detect the true sample using the Raman spectrum, which spans from 450 to 1050 cm⁻¹, the milk powder samples were fortified with 3, 5, and 10% melamine in comparison to a blank. Using PLS modeling, the detection limit of melamine at the Raman peak positions of 673 and 982 cm⁻¹ was 0.13 percent (Cheng Y *et al.*, 2012) ^[95]. Enhancements in Raman signal processing enable the detection of melamine in even minute

quantities. Fluorescent molecules in the sample may have an impact on the spectra that are produced, which could impede the accurate depiction of the findings. The incorrect interpretation of the results could stem from improper handling of the very sensitive instruments or from improper knowledge of how to evaluate the data that has been gathered.

6.6 UV-Vis and fluorescence spectroscopy

One of the most used food analytical techniques that measures the quantity of light absorbed by the samples is UV-Vis spectroscopy. The main advantages of this spectroscopy are its simplicity of use, ability to identify a wide range of substances, non-destructive nature, high precision, improved reproducibility, and modest discernment. This method is based on Beer's law, which says that the quantity of an analyte dissolved in a solvent is exactly equal to the quantity of light absorbed. Fluorescence is the process by which fluorescent substances, or fluorophores, release light after absorbing UV or VIS radiation. Compounds with several conjugated bonds carrying aromatic rings and linear structures are known as fluorophores. The apparent fluorescence intensity and spectrum pattern are significantly influenced by the sample illumination geometry and optical density. The most common geometry used in fluorescence spectroscopy is the right-angle observation of a centrally lit cuvette. However, when analyzing viscous samples, it can be challenging to perform right-angle measurements. Another problem with this method is that the optical properties of living materials, like meat or seafood, may cause the fluorescence's intensity to decrease as path and reabsorption increase (Airado-Rodríguez D *et al.*, 2014) ^[97].

Front-face fluorescence spectroscopy, which causes very little fluorescence spectral distortion during both emission and absorption, can be used to overcome these problems. The fluorescence emission is collected from the same area at an angle that minimizes reflected and scattered light, while the excitation light is focused on the sample's front surface. This technique is effective with solid, opaque, or concentrated samples (Albani JR *et al.*, 2008) ^[98]. It was evaluated if fluorescence spectroscopy could discriminate between frozen-thawed and fresh sea bass (*Dicentrarchus labrax*) samples (Karoui R *et al.*, 2017) ^[99].

The four fluorophores that were used were NADH (340 nm), tryptophan (290 nm), riboflavin (380 nm), and vitamin A (410 nm). The outcomes demonstrated that the technique could distinguish between fresh and frozen-thawed sea bass samples, as well as changes in the quality of different frozen fish samples before preservation.

Seventy-two of the 78 samples were correctly classified after factorial discriminant analysis (FDA) was applied to the spectra obtained following excitation sets at 340 and 380 nm. Thus, it can be concluded that the use of fluorescence spectroscopy in combination with chemometric devices could provide a reliable means of differentiating between materials that are fresh and those that have thawed. Numerous chemical and rheological characteristics were effectively used in conjunction with fluorescence spectroscopy to distinguish between various beef muscles (Sahar A *et al.*, 2009) ^[100].

Fluorescence spectroscopy in the 305-700 nm series was used to distinguish seven bovine muscles. Emission spectra were produced by varying the excitation wavelength at 290,

322, and 382 nm. Using fluorescent spectra, various muscles were categorized according to their dry matter content, protein, fat content, and collagen content. Similar potential exists for selecting and representing beef muscles (66 samples) using fluorescence spectroscopy. These muscles include the Semitendinosus ($n = 24$), Rectus abdominis ($n = 24$), and Infraspinatus ($n = 18$). The data is then treated using PLS-DA and PLS regression models. 305-400, 340-540, and 410-700 nm were the ranges in which emission spectra were recorded, with the excitation wavelengths fixed at 290, 322, and 382 nm, respectively. For three muscles, an excellent classification of 100% was attained. For the three muscles, discriminant analysis applied to emission spectra in the 410-700 and 340-540 nm ranges produced 96 and 90% of good classification, respectively. The investigation's conclusions shown that there is great potential for separating between beef muscles expending fluorescence spectroscopy and chemometrics (Sahar A *et al.*, 2014) ^[101]. Similar findings were later made by (Ait-Kaddour A *et al.*, 2018) ^[102], demonstrating this spectroscopy's capacity to carry out muscle sample discrimination. This spectroscopic method hasn't been applied in many investigations to distinguish between fresh and frozen-thawed beef.

Furthermore, Ruoff, Luginbühl (Ruoff K *et al.*, 2006) ^[103] used front-face fluorescence spectroscopy to confirm the identity of unifloral and multifloral honey varieties that had been previously categorized using traditional techniques like chemical, pollen, and sensory tests. PCA and LDA were used in the analysis of the spectral data. Front-face fluorescence spectroscopy was found to be an efficient method for determining the ethnic origins of honey and confirming its provenance within a comparable unifloral honey variety.

7. Innovations in sensor technologies

7.1 Passive sensors

A variety of technologies constitute the modern production system, but sensors, actuators, effectors, controllers, and control loops are the primary technologies employed [Herrojo, C. *et al.*, 2019] ^[104]. In a smart factory, sensors are essential because they gather and apply precise data to the production processes, improving the quality of the final product. Electrical, opto-electrical, or electronic devices with sensitive elements within are called sensors. They are used to find out whether a certain object or function is present [Bibby, L & Dehe, B., 2018] ^[105, 112, 133].

A physical input is frequently converted by sensors into an electrical signal that may be assessed and analyzed to inform judgments regarding the actions being performed [Lass, S. *et al.*, 2020] ^[106]. Manufacturers now have unprecedented control and data acquisition capabilities because to recent advancements in sensor technology. Sensors have two modes of operation: active and passive [Mulloni, V & Donelli, M., 2020] ^[107, 129].

The sensor needs a specific physical stimulation in order to function when it is actively running. For instance, color identification sensors work because they require an object to be illuminated by visible light in order for the sensor to feel a physical input. The physical stimulus does not need to be given in the passive case because it already exists [Mulloni, V. & Donelli, M., 2020] ^[107, 129]. For instance, infrared devices are passive as the body's temperature already generates infrared radiation, which serves as the stimulus.

Numerous sensor types have been created and effectively applied to industrial process control. A range of sensor types are used in smart factories, ranging from simple humidity and temperature monitoring to complex location and product sensing [Jeon, B *et al.*, 2020] ^[108]. By assisting with advanced factory activities like product movement, robotic and milling process management, and environmental element sensing, these sensors improve manufacturing efficiency. In a factory setting, the primary parameters for measurement and control are flow, force, position, temperature, and pressure [Landaluce, H *et al.*, 2020] ^[109, 125].

7.1.1 Temperature sensors

Since temperature has an instantaneous impact on the qualities of materials and products, it is one of the most crucial parameters in industrial operations to monitor and control. A temperature sensor is a device that can collect temperature data from an external source and then convert it into a format that can be read by another device. [Le, D.N *et al.*, 2019] ^[110, 130].

Liquids, solids, and gases can all have their thermal characteristics measured using these sensors. Many chemical and electrically resistant temperature sensors have been developed in recent years. There are two types of these sensors:

- (1) Low-temperature sensors, which detect temperatures between 100 and +400 C using substances like liquid crystals, semi-conductors, and phosphors
- (2) High-temperature sensors that detect temperatures between 500 and 2000 C using blackbody radiations [Kadechkar, A *et al.*, 2020] ^[111].

7.1.2 Pressure sensors

Variations in pressure can be recorded by pressure sensors and translated into an electrical signal, the strength of which is dictated by the pressure being applied. These electro-mechanical devices [Bibby, L & Dehe, B *et al.*, 2018, Sattar, H *et al.*, 2019] ^[105, 112, 113] sense force in gases or liquids and provide control signals to display devices. These sensors can also be used to detect changes in the atmosphere [Zuo, G. *et al.*, 2018] ^[114, 131]. In order to forecast weather patterns and fluctuations, for example, barometric pressure sensors can detect changes in the atmosphere. Another example would be vacuum sensors, which are used when the air pressure in a vacuum is exceeded a condition that might be difficult to detect using mechanical methods.

7.1.3 Position sensors

These sensors detect the locations of valves, doors, throttles, and other equipment. The ability to track the precise positions of tools, work-in-progress, and other items crucial to production within the facility is made easier by these sensors [Islam, M.M.M *et al.*, 2018, Petrov, R.V *et al.*, 2020] ^[115, 116]. Motion and proximity sensors work similarly to position sensors to detect movement of an object and initiate actions such as turning on a floodlight or detecting when an object is within a sensor's range [Gödecke, M.L *et al.*, 2020, Chan, Y.J *et al.*, 2020] ^[117, 118].

7.1.4 Force sensors

According to Xu, L. *et al.* (2020) ^[119] and Cherkun, A. *et al.* (2020) ^[112], the function of force sensors is to transform applied forces, such as compressive and tensile forces, into electronic signals that indicate the force's magnitude. Following transmission, these signals reach computers,

controllers, or indicators, which either inform operators of the activities taking place or serve as inputs for controlling machinery and processes. In smart factories, several force sensors are used, depending on the type of force being detected [Nastro, A. *et al.*, 2020] ^[121]. For instance, force sensing resistors detect the rate at which an applied force varies, load cells measure compressive forces, and strain gauges measure internal resistance forces.

7.1.5 Flow sensors

These sensors are capable of identifying the movement of gases, liquids, or solids through a conduit or pipe. These sensors have several uses in the processing sectors and allow machinery to run as efficiently as possible [Zang, H. *et al.*, 2019] ^[122]. A flow sensor uses ultrasonic technology to measure flow and can be partially mechanical or electronic [Garrett, A. *et al.*, 2020] ^[123]. For instance, automobile flow sensors adjust fuel distribution to the fuel injectors based on engine air intake and monitor it to provide the engine the optimum fuel possible. Flow sensors are also used in medical ventilators, where patients need the right amount of oxygen and air delivery to breathe [Bekraoui, A. Hadjadj, A., 2020] ^[124].

7.2 Smart sensors

Smart sensors have attracted attention because of their wide range of applications and potential relevance, coupled with other recent technical breakthroughs. Due to the integration of computing and IoT into industrial processes, common sensors have become "smart". Because of this, they are now

able to use the data they have collected to perform complex calculations [Landaluce, H *et al.*, 2020] ^[109, 125].

Smart sensors, with their improved capacities and remarkable compactness and adaptability, have turned heavy machinery into intelligent intelligence. These days, smart sensors are gadgets with embedded algorithms, digital interfaces, signal conditioning, and detection and self-awareness capabilities [Choy, J.L.C *et al.*, 2020; Kim, J *et al.*, 2019] ^[126, 127]. These sensors are made to function as Internet of Things (IoT) components, converting data into digital form in real time so that it may be transmitted to a gateway [Dahlin, A.B., 2012] ^[128].

These characteristics allow intelligent sensors to predict, monitor, and respond to situations in real time. The majority of sophisticated, multi-layered operations that intelligent sensors are employed for include gathering raw data, adjusting sensitivity and filtering, motion detection, analysis, and communication [Mulloni, V *et al.*, 2020] ^[107, 129].

Wireless sensor networks (WSNs), in which the sensor nodes are networked with one or more additional sensors and sensor hubs to establish a sort of communication technology, are one use case for smart sensors. Additionally, information from many sensors can be combined to deduce conclusions about a problem that is already present; for instance, data from temperature and pressure sensors may be used to predict the beginning of a mechanical breakdown. While Figure 2 shows the elements of a typical smart sensor, Figure 1 summarizes the key features of smart sensors [Le, D.N *et al.*, 2019] ^[110, 130] [Zuo, G *et al.*, 2018] ^[114, 131].

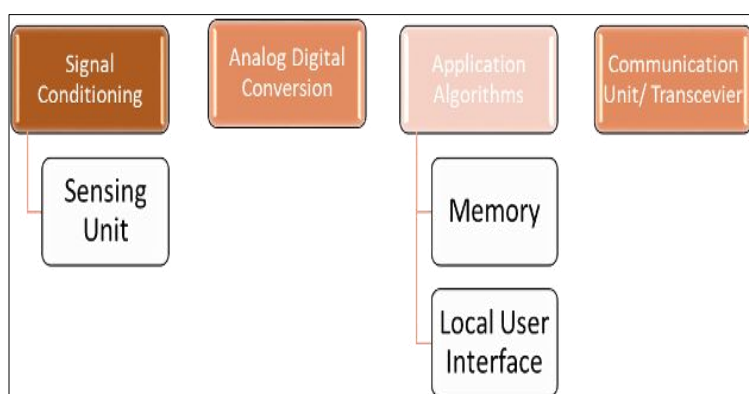


Fig 1: Smart sensor building blocks

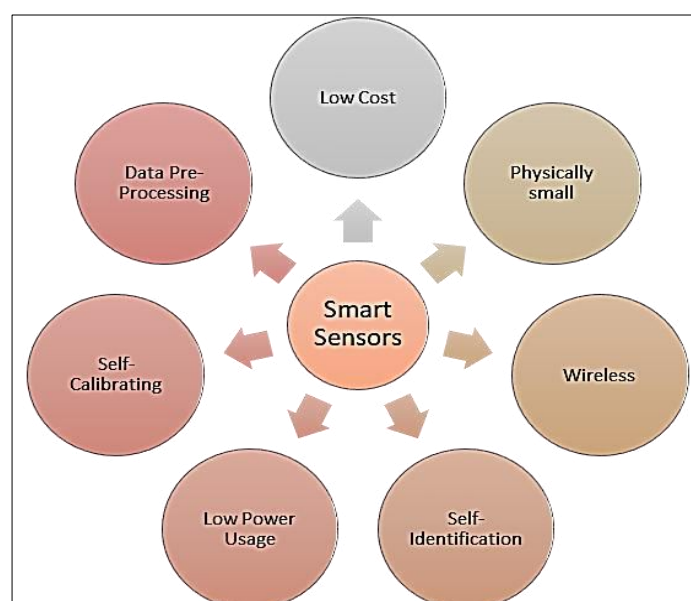


Fig 2: Features of a smart sensor

7.3 Nuclear sensors

According to Li, J. *et al.* (2016) ^[132], nuclear sensors are extremely rare because of two factors: their high cost and stringent safety requirements. The availability of low-level radiation sources for the safe use of these sensors has improved recently [Bibby, L & Dehe, B.,2018] ^[105, 112, 133].

7.4 Micro-sensors (MEMS Sensors)

Micro-machined structures in two and three dimensions are found in microelectromechanical system (MEMS) devices [Li, J. *et al.*, 2016] ^[132]. Since these sensors transform mechanical signals from an energy source into electrical form, they can be thought of as tiny transducers. Industries are currently making successful use of sensors that detect a variety of characteristics, including temperature, pressure, force, speed, sound, magnetic field, optical, biological, and chemical [Bibby, L & Dehe, B.,2018] ^[105, 112, 133].

7.5 Nano-sensors (NEMS)

The most recent advancement in sensing technology are nano-sensors, which are based on nanotechnology [Kim, J *et al.*, 2019] ^[127]. These belong to the class of devices known as nano electromechanical systems (NEMS), which also contain nano-actuators.

Case studies and real-world applications

There are two primary analytical techniques that can be used to detect fraud: the targeted method, which seeks to identify and/or quantify certain analytes that may be used as indicators of a fraudulent or real product. The second strategy, known as the untargeted approach, focuses on characterizing the pattern of chemical composition and/or physical attributes overall on a global scale (Rodionova 2020).

Soft Independent Modeling of Class Analogy is the most used OCC technique for PCA decomposition-based food authenticity (SIMCA). Svante Wold offered the first version and it is currently a common moniker for a whole set of techniques (Wold,1976).However, new chemometrics methods are being created, and those that already exist and are sufficiently general and grounded in mathematical concepts are being refined to enable them to be used for various scenarios. Chemometrics has been utilized extensively in the literature as a crucial instrument for non-destructive methods to evaluate a variety of situations, including rice differentiation, beer classification, and sensory assessment (Szymańska *et al.*,2015).

The change in the food sector has coincided with a gradual development and notable advancement in the application of food metabolomics in recent years (Clish & C. B, 2015) (Kim *et al.*,2016) ^[127] (Yan *et al.*,2018).There have been notable outcomes from the use of metabolomics in food commodities for authentication (Erban *et al.*,2019).The foundation of NIR is the absorption of electromagnetic energy, or light, in the 780-2500 nm wavelength range (Esteki *et al.*,2018).Each food item has a unique spectrum

that serves as a fingerprint for identification and categorization (Barbin *et al.*,2015).

Chromatographic techniques are among the many approaches that have been created employing advanced and effective technologies to identify food fraud in food (Esteki *et al.*,2018).Genomics-based techniques, metabolomics, and proteomics (Bohme *et al.*,2019) (Ortea *et al.*,2016) and methods for spectroscopy (Abbas *et al.*, 2018).

The emergence of portable spectrometers is a benefit of spectroscopic techniques (Zhu *et al.*,2022) ^[144].Thus, methods like DNA metabarcoding that are based on High Throughput Sequencing Technologies (HTS) can satisfy this criteria (Galimberti *et al.*,2015) ^[148] (Bruno *et al.*,2019).For instance, these methods have been effective in determining the ingredients in honey (Hawkins *et al.*,2015), Herbal teas and spices(Frigerio *et al.*,2021), intricate matrices like hamburgers(Mottola *et al.*,2022), however also for innovative insect-based food quality control(Frigerio *et al.*,2020).

Future trends and prospects in food authentication:

Food authentication is a crucial issue, and there is a need to make a coherent plan that requires a cautious choice for the finest sample processing approach, an acceptable analytical procedure, an obvious metabolite identification, and an authentic neutral chemometric analysis (Granato *et al.*,2018) (Bohme *et al.*,2019) introduced a thorough study of the latest method for the determination of food authenticity based on DNA.

For food authentication, different instrumental techniques are being proposed that include trace element analysis, NMR spectroscopy, HPLC, stable isotopic ratio analysis, and the most latest approaches of proteomics, metabolomics, and genomics (Drivelos *et al.*,2012) ^[2] (Ortea *et al.*,2012) (Cubero-Leon *et al.*,2014) ^[4]. The breakdown of DNA is a major drawback of DNA methods in food authentication that can overcome by using peptide-based methods (Buckley *et al.*,2009).

Some restricting aspects faced by food analysis labs is their limited accessibility to source materials, taking into account to broad range of feasible observers, mass, and matrix mixture of original samples to be analyzed (Emons *et al.*,2006).Latest public scandals, like the China melamine crisis in 2008 and in 2013 horsemeat scandal in Europe have focused attention on the requirement of enhanced food authenticity identification methods (Kowalska *et al.*,2018).

Conclusion

Food safety and quality assurance are changing due to the developments in DNA-based authentication methods as PCR, NGS, and DNA Barcoding. These approaches offer strong, accurate, and dependable instruments for confirming food product authenticity, identifying adulteration, and guaranteeing regulatory compliance. The use of these DNA-based methods to protect consumer health and uphold industry confidence will only increase as food supply chains get more intricate, making them essential to the quest for high-quality and safe food.

Table 3: Types of Sensors, Characteristics, Materials, Applications, Advantages, and Disadvantages

Sensor Type	Characteristics	Material	Uses	Advantages	Disadvantages	References
Nuclear sensor	Function according to the concepts of optical sensors, in which a medium enables the radiation transmission amid a detector and a source; as well as the extent of transmission is lessened in accordance with the measured parameter	The gamma ray source is Cesium 137, while the gamma ray detector is a sodium diode device.	Measurement of mass flow and medical scanning applications	Carbon emissions are zero, and energy is independent	incredibly costly and vulnerable to background radiation contamination	(Li, J <i>et al.</i> , 2016) ^[132]
Micro-sensors	A mechanically functioning part is combined with microelectronic components. The usual dimensions of these sensors ranging from 0.01 mm to 5 mm to 10-5 m.	semiconductor substance made of silicon, occasionally manufactured with metals gases, polymers, and plastics as well as ceramics placed on the silicon foundation	Mostly utilized in the automotive and medical apparatus, like blood pressure quantification.	Reduced dimensions; enhanced functionality; increased dependability; reduced expenses of production.	Possess a low capacitance and output signals that are easily contaminated by noise; generate output signals for relatively low magnitude.	(Li, J <i>et al.</i> , 2016) ^[132]
Nano-sensors	Vary in size utilizing nanotechnology, ranging from 1 to 1000 nm.	thin semiconductor or metal sheet layers; more sophisticated than MEMS utilizing unique techniques for etching, optical printing, or electron beam lithography	Applied as biological sensors, accelerometers, and sensors for aircraft substances.	Reduced size, lower production costs, and lower power usage	Challenging to manage; transient noise problems.	(Li, J <i>et al.</i> , 2016) ^[132]

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