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Review article

Advances in detection technology for authentication of vegetable oils: A comprehensive review

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ABSTRACT

Biomarkers are specific indicators that can be used to authenticate vegetable oils by reflecting unique characteristics such as variety or geographical origin. Biomarkers can originate from the primary components of the vegetable oil itself or from contaminants and trace substances linked to processing methods or adulterants. The review highlights the key findings in the identification of novel biomarkers for vegetable oil authentication. Various analytical techniques have proven effective in distinguishing unique biomarkers associated with specific vegetable oil varieties or geographical origins. The use of biomarkers of vegetable oils and associated contaminants or trace substances offers a comprehensive approach to authentication. However, the identification of novel biomarkers holds immense potential for enhancing food safety, preventing fraud, and safeguarding consumer health in the vegetable oil industry. The ongoing research and advancements in biomarker identification represent a promising avenue for addressing authenticity concerns in vegetable oils.

1. Introduction

The rising rates of adulteration, mislabelling, and food fraud in recent years have made the authentication of vegetable oils a crucial problem. As consumers' nutritional habits and health consciousness have grown, so too has the need for vegetable oils. As a result, dishonest business practices have turned their attention to the vegetable oil sector, bringing inferior or fake goods into the supply chain [1]. The creation and application of efficient techniques for the quality assurance and certification of vegetable oils are urgently needed in order to address this problem. Finding innovative biomarkers that can be used as trustworthy indications to confirm the

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provenance, calibre, and purity of vegetable oils is one possible strategy. Biomarkers are particular molecules or substances that can be used to determine the authenticity or quality of a biological sample. Examples of these samples include vegetable oil [2]. These biomarkers can come from a variety of sources, such as the main ingredients of the vegetable oil itself, pollutants, or traces of compounds connected to certain processing techniques or adulterants.

The food industry is highly concerned about lipid peroxidation as a result of the growing trend of substituting monounsaturated and polyunsaturated fatty acids—which are regarded as healthier by consumers—for hydrogenated oils containing saturated fatty acids. However, these fatty acids are far more prone to oxidation than SFAs. Furthermore, deep-frying or cooking temperatures can cause oils high in MUFAs and PUFAs to produce RCCs, which start as ALEs and offer major health hazards. While it is highly recommended to consume oils high in MUFAs and PUFAs, these oils must first be "protected" before being used in cooking [23]. Certain oils, like olive oil, are naturally protected by their high endogenous antioxidant content (polyphenols and tocopherols), while other oils, like soybean, sunflower, and peanut oils, require the addition of exogenous antioxidants during production to increase their resistance to oxidation because of the refining process.

Advanced analytical techniques like nuclear magnetic resonance (NMR), mass spectrometry, chromatography, and DNA-based technologies are used to identify biomarkers for the authentication of vegetable oils. These methods make it possible to identify and measure individual molecules or chemical signatures that are exclusive to a given type of vegetable oil or region of origin [24]. The wide range of vegetable oil varieties on the market presents a significant obstacle in the search for biomarkers for vegetable oil verification. Many different types of plants, such as olive, soybean, sunflower, coconut, and palm, can be used to make vegetable oils. Since every type of oil has a unique chemical profile and composition, it is crucial to find certain biomarkers that can distinguish between various types of vegetable oils [25].

Molecular markers that reflect a typical food state or condition are known as novel biomarkers, and they enable more accurate product discrimination [26]. Biomarkers are heavily utilised in the field of nutrition and health research for a variety of reasons. These include biomarkers for nutrient status and dietary intake, biomarkers for measuring the biological effects of certain food ingredients, and biomarkers for evaluating the impact of diet on health.

Moreover, a great deal of validation and cross-validation research is needed to create strong and trustworthy biomarker detection techniques. To determine the specificity and sensitivity of the discovered biomarkers, a vast number of samples from various sources and geographical locations are analysed in these investigations. To guarantee the stability and repeatability of the discovered biomarkers, other aspects including shelf life, processing techniques, and storage conditions must be taken into account. There are many significant uses for the discovery of new biomarkers for vegetable oil authenticity [27]. First off, quality control procedures and rules can be enforced by regulatory bodies and food testing facilities using these biomarkers, which will stop the sale and distribution of fake or contaminated vegetable oils. Second, they can help with the labelling and traceability of products made with vegetable oil, which will boost customer confidence and guarantee supply chain transparency. In conclusion, a vital area of study that has attracted a lot of attention lately is the identification of novel biomarkers for the verification of vegetable oils. The need for precise and trustworthy techniques to identify adulteration and guarantee product quality is growing as the market for vegetable oils continues to expand. The creation and verification of biomarkers for vegetable oil authenticity have enormous promise for improving food safety, stopping

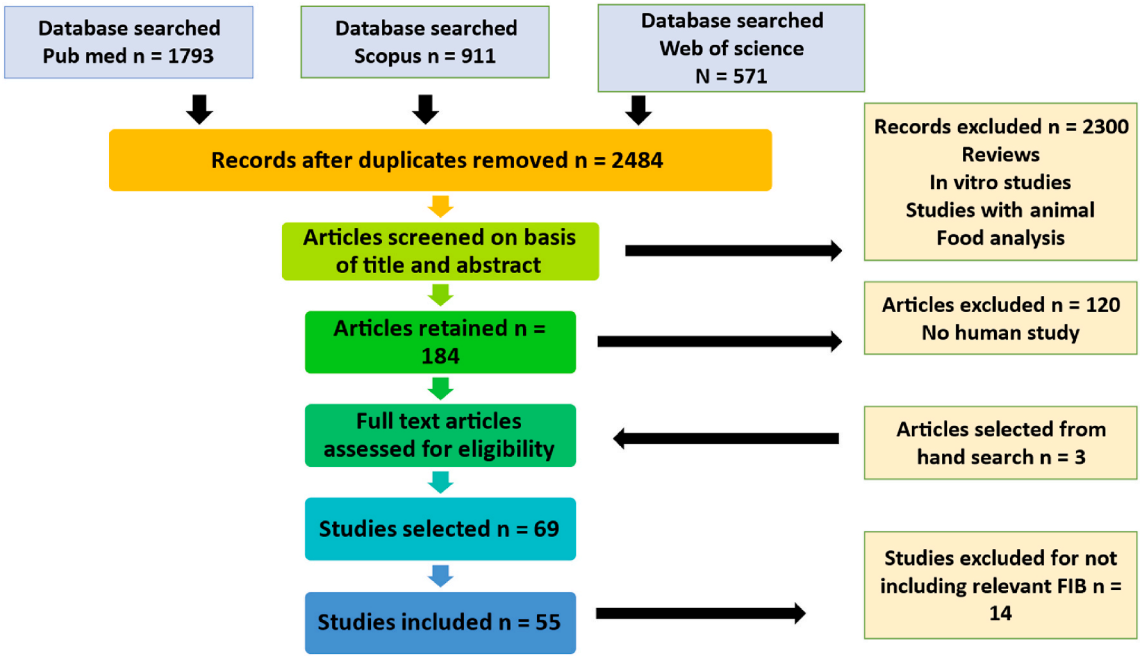

Fig. 1. Flow diagram of study selection.

fraud, and eventually protecting the health of consumers. This review objectives are to examine how biomarkers function in the authentication of vegetable oils, outline the sources of biomarkers, such as primary components and contaminants, and highlight current developments in the identification of new biomarkers for authentication. Also to clarify how well different analytical methods distinguish distinct biomarkers linked to particular types of vegetable oil or regions of origin. It also emphasises the all-encompassing way that biomarkers provide for vegetable oil authentication, which includes both inherent qualities and extrinsic elements like pollutants and processing techniques. In addition, the analysis seeks to highlight how innovative biomarkers in the vegetable oil sector may improve food safety, fight fraud, and protect consumer health. The purpose is to provide light on the continued investigations and developments in biomarker identification as a possible solution to the authenticity issues with vegetable oils.

2. Techniques for vegetable oil authentication

Vegetable oil authentication techniques employ a range of analytical techniques to identify and measure particular chemical profiles or biomarkers that are indicative of the oil's provenance, authenticity, and quality. These methods are crucial for protecting the purity of products containing vegetable oil and averting food fraud and adulteration. To improve the precision and dependability of vegetable oil authentication, these methods can be applied singly or in combination. To guarantee these techniques' resilience and suitability for use in real-world scenarios, a significant number of genuine samples from various sources and geographical areas must be used for validation and cross-validation. For the analysis of vegetable oil products to be consistent and trustworthy, it is also essential to build reference databases and standardized processes for vegetable oil authenticity [28]. The flow diagram of study selection for the review is presented in Fig. (1). Biomarker and its identification purpose in vegetable oil composition is presented in Table 1.

2.1. DNA barcoding

Based on an organism's DNA sequence, DNA barcoding is a potent tool for organism authentication and identification. It entails the amplification and examination of particular genomic areas known as DNA barcodes, which differ throughout species but are highly conserved within a given taxonomic group. In addition to being widely used in the fields of biodiversity and species identification, DNA barcoding is now being used more and more to verify the authenticity of food items, such as vegetable oils [29]. The application of DNA barcoding for the certification of vegetable oil requires the selection of particular DNA target regions. Target region selection is influenced by a number of variables, including taxonomic coverage, sequence variability, reference database accessibility, and practical concerns. The internal transcribed spacer (ITS) region of the ribosomal DNA gene and the trnL-trnF intergenic spacer region of the chloroplast genome are the most often employed target regions for vegetable oil authenticity [30]. There are various steps in the DNA barcoding process: DNA Extraction, PCR amplification, DNA sequencing, Sequence Analysis and Identification, Database Creation and Validation are the steps that are involved in DNA barcoding process. Extracting DNA from the sample of vegetable oil is the first step. There are various techniques for extracting DNA; these include commercial kits, miniaturised fast techniques, and CTAB-based techniques. Ensuring that the extracted DNA is of superior quality and devoid of impurities that may impede further analysis is imperative [14]. The target DNA region of interest is precisely amplified using the polymerase chain reaction (PCR). The purpose of PCR primers is to bind to conserved areas that surround the target region. Following amplified DNA fragmentation, sequencing can begin [3].The PCR products are delivered to next-generation sequencing platforms or conventional Sanger sequencing for DNA sequencing. The extracted DNA sequences are compared and matched with reference sequences found in DNA databases like GenBank and the Barcode of Life Data Systems (BOLD) [31]. Using a variety of bioinformatics techniques, the acquired DNA sequences are examined and contrasted with reference sequences. There are various techniques available for sequence comparison, such as BLAST (Basic Local Alignment Search Tool). The degree of identity or similarity to well-known DNA sequences permits the species of vegetable oil to be identified and validates its legitimacy [32]. By adding verified reference sequences from verified vegetable oil samples of recognized species, a comprehensive DNA barcode library tailored to vegetable oils can be created. Vegetable oil authenticity can be made more accurate and dependable by adding new species and varieties to the database, which is constantly being updated. There are various benefits of using DNA barcoding for the verification of vegetable oil. It offers a high-throughput, quick, and precise way to determine the botanical source of vegetable oils, independent of their forms or processing techniques. Additionally, it can identify genetically modified organisms (GMOs) or other adulterants in the vegetable oil supply chain, protecting the safety and integrity of the final product [33]. However, using DNA barcoding for vegetable oil authenticity presents a number of difficulties. One of the difficulties is the existence of deteriorated or low-quality DNA in the oil samples, which could have an impact on the

Table 1
Biomarker and its identification purpose in vegetable oil composition.

Biomarker	Purpose of Identification	Importance	Reference
Fatty Acid Composition	Distinguishing different vegetable oil sources	High	[3]
Sterol Composition	Determining the presence of adulterants or contaminants	Medium	[4]
Triacylglycerol Structure	Authenticating specific vegetable oil varieties	Medium	[5]
Wax Ester Content	Discriminating between refined and unrefined oils or different vegetable oils	Low	[6]
Tocopherol Content	Determining the freshness and quality of vegetable oils	Low	[7]
Pigment Content	Authenticating specific vegetable oil varieties or detecting adulterants	Low	[8]

dependability and performance of DNA amplification and sequencing. Furthermore, in order for DNA barcoding to correctly identify the vegetable oil species, access to thorough and carefully curated reference libraries is necessary. Therefore, in order to enhance the coverage of vegetable oil species, ongoing efforts are required to grow and enhance these databases [8]. In conclusion, DNA barcoding is a practical technique for confirming the legitimacy of vegetable oils. By concentrating on certain DNA regions, it enables the identification of various vegetable oil species, the detection of adulteration, and the verification of a product's provenance. To ensure that DNA barcoding for vegetable oil authenticity is accurate and reliable, reference databases and standardised procedures must be established and used [34].

2.2. Mass spectrometry

Vegetable oil quality control and verification have been shown to be much aided by the sophisticated analytical method known as mass spectrometry (MS). It makes it possible to identify and measure particular molecules or biomarkers, which in turn makes it possible to determine the oil's overall quality, adulteration, and botanical origin. Vegetable oil authentication frequently uses a number of mass spectrometry (MS)-based methods, including as direct infusion techniques, liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS) [35].

2.2.1. Gas chromatography-mass spectrometry (GC-MS)

Vegetable oil volatile and semi-volatile chemical analysis is a common application for GC-MS. This method separates volatile chemicals according to their partitioning capabilities by first subjecting the oil sample to gas chromatographic separation. The mass-to-charge ratios of these molecules are then ascertained by ionising and fragmenting them inside the mass spectrometer. Using GC-MS, a variety of volatile chemicals found in vegetable oils, including triglycerides, sterols, tocopherols, and volatile fragrance compounds, can be identified and quantified. These chemicals' distinctive profiles can be utilised as biomarkers to identify adulteration or verify the identity and distinction of different vegetable oil kinds [36].

In GC, the sample is vaporised and injected into a gas chromatography column. A stationary phase that is packed into the column divides the sample components according to variations in their polarity, volatility, and other physicochemical characteristics. The sample is carried through the column by the mobile phase, which is usually an inert gas such as helium. After separation, the individual sample components enter the mass spectrometer. Here, the ion source—which may be chemical ionisation (CI) or electron impact (EI)—ionizes them. EI entails bombarding the molecule with high-energy electrons, which breaks it up into charged fragments. In CI, ions are produced through a reaction with reagent gas. The ions are accelerated through an electric field and separated according to their mass-to-charge ratio (m/z) by a mass analyzer, usually a quadrupole or time-of-flight (TOF) analyzer [26]. By counting the ions that reach it, the detector generates a mass spectrum. Uses: GCMS is extensively used in environmental analysis to detect and quantify pollutants, such as pesticides, volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs), in air, water, soil, and food samples. It is employed in the investigation of pollution sources, environmental monitoring, and the evaluation of remediation strategies' efficacy. GCMS plays a crucial role in forensic analysis for the identification of drugs, explosives, arson residue, and toxic substances. It can offer vital proof in toxicological tests and criminal investigations. GCMS is utilised in pharmaceutical analysis for drug discovery, quality control, and pharmaceutical research [30]. It can evaluate drug metabolic pathways, identify the active ingredients in medications, find contaminants, and assess the stability of drug formulations. GCMS is employed in the analysis of food and beverage samples to detect contaminants, residues, additives, and flavors. It aids in ensuring food safety, figuring out nutritional value, and assessing the calibre and legitimacy of food items. GCMS is widely used in the petrochemical industry for the analysis of hydrocarbons, pollutants, and additives in crude oil, gasoline, and other petroleum products. It helps with refining process optimisation, contamination source identification, and fuel quality monitoring. GCMS is utilised in the analysis of natural products, such as essential oils, herbal extracts, and flavors. It facilitates the identification and measurement of the chemical components included in these goods, allowing for formulation development, stability testing, and quality control. These are only a handful of the many uses for GCMS that exist. The method is very adaptable and useful in many scientific fields, such as chemistry, medicine, environmental science, and more [25].

2.2.2. Liquid chromatography-mass spectrometry (LC-MS)

Mass spectrometry and liquid chromatography are combined in LC-MS to identify and quantify chemicals. Liquid chromatography separates compounds based on their interaction with a stationary phase. It works especially well for analysing polar and non-volatile substances found in vegetable oils. Many different molecules, such as polar lipids, phospholipids, diacylglycerols (DAGs), triacylglycerols (TAGs), free fatty acids, sterols, and other minor components, can be identified and quantified using LC-MS. These substances may function as further biomarkers for vegetable oil authenticity [34].

Another potent analytical method that combines the concepts of mass spectrometry with liquid chromatography is called Liquid Chromatography Mass Spectrometry, or LCMS. A liquid solvent is used to dissolve the sample before it is put into a liquid chromatography column. The stationary phase in the column—a polymer or bonded silica material, for example—separates the sample's constituent parts according to variations in their polarity, sizes, and other physicochemical characteristics. The material is passed through the column by a liquid mobile phase. The separate components of the sample are then introduced into the mass spectrometer. In LCMS, many ionisation methods are used, such as air pressure chemical ionisation (APCI) and electrospray ionisation (ESI). In ESI, the sample solution produces charged droplets, but in APCI, the sample reacts with ions produced during a corona discharge. A mass analyzer, such as a quadrupole or a time-of-flight (TOF) analyzer, separates the ions created in the ion source according to their mass-to-charge ratio (m/z), much like in GCMS. The ions are accelerated through an electric field. By counting the ions that reach it, the

detector generates a mass spectrum. To detect and measure the proteins and metabolites present in biological samples, LCMS is frequently employed in proteomics and metabolomics research. It helps with understanding illness mechanisms, finding biomarkers, and researching biological processes. To determine the concentration of medications and their metabolites in biological fluids like blood or urine, pharmacokinetic investigations use LCMS. Drug dosing and treatment regimen optimisation are aided by its ability to ascertain drug absorption, distribution, metabolism, and excretion (ADME) characteristics. In environmental analysis, LCMS is used to identify and measure a variety of contaminants, such as endocrine disruptors, pesticides, herbicides, medicines, and personal care items. It supports the monitoring of environmental pollution and the evaluation of its effects on ecosystems and public health. When analysing food samples to find and measure pollutants, additives, residues, and poisons, LCMS is an essential tool. It is employed in the process of identifying and verifying the existence of mycotoxins, allergies, veterinary medications, and other hazardous materials in food items. When it comes to forensic analysis, LCMS is used to find and identify drugs, illegal substances, and their metabolites in biological samples like urine or blood. It helps with post-mortems, toxicology testing, and criminal investigations. Plant extracts, herbal remedies, and nutritional supplements are examples of natural items that are screened and profiled using LCMS. It supports these items' standardisation, purity evaluation, and quality control by assisting in determining their chemical makeup. These are only a handful of the many uses for LCMS that exist. The method is very adaptable and useful in many scientific domains, such as forensics, food science, environmental science, and medicines.

2.2.3. Direct infusion mass spectrometry

Vegetable oil samples are directly injected into the mass spectrometer in direct infusion mass spectrometry, bypassing any preliminary chromatographic separation. With this method, vegetable oils may be quickly and efficiently analysed in large quantities, yielding insightful data on the abundances and profiles of individual compounds. To ionise the substances contained in the oil, direct infusion mass spectrometry can be used with various ionisation methods like air pressure chemical ionisation (APCI) or electrospray ionisation (ESI). This makes it possible to identify and measure a large variety of lipid and non-lipid components, giving important information about the make-up and legitimacy of the vegetable oil [8]. Vegetable oil authentication can benefit from various advantages provided by mass spectrometry techniques. Even at trace levels, they offer excellent specificity, sensitivity, and accuracy in the identification and measurement of substances. These approaches are flexible and can be applied to the examination of many kinds of vegetable oils, regardless of how they are processed or formulated. Furthermore, MS-based methods can aid in the detection of pollutants, adulterants, or chemicals associated to processing, which helps in the evaluation of vegetable oils' overall quality and safety. However, access to extensive reference databases and the creation of analytical techniques tailored to particular oil types and markers are necessary for the successful application of mass spectrometry for vegetable oil verification [36]. To verify the quality and dependability of the results, the data from the MS analysis must also be evaluated and confirmed using the proper statistical methods and reference samples from verified vegetable oils. To sum up, mass spectrometry methods including GC-MS, LC-MS, and direct infusion MS provide useful instruments for vegetable oil quality control and authenticity. These approaches evaluate the source, purity, and general quality of vegetable oils by identifying and quantifying particular biomarkers or molecules. This allows for the detection of adulteration and ensures consumer safety and confidence [37].

2.3. Near-infrared spectroscopy (NIRS)

Vegetable oil authentication has been made easier with the use of near-infrared spectroscopy (NIRS), a quick and non-destructive analytical method that has gained favour recently. It makes use of the way molecules in the oil interact with near-infrared light to create a distinct spectral fingerprint, which is subsequently examined to reveal details about the oil's composition and quality. Numerous benefits come with NIRS, including speed, ease of use, affordability, and the capacity to analyse a big number of samples without requiring a lot of sample preparation [10]. The basis of the NIRS method is the idea that certain oil molecules absorb near-infrared light in particular ways, giving rise to distinct absorption wavelengths. These absorption bands enable for the identification and measurement of particular substances since they correlate to different chemical bonds and functional groups found in the oil. A cuvette or other appropriate container is filled with a representative sample of vegetable oil in order to conduct NIRS analysis. After that, the sample is exposed to near-infrared light. The transmitted or reflected light is then measured using a spectrometer that has a detector that can identify near-infrared wavelengths. Following that, chemometric models and statistical methods are applied to the spectral data in order to estimate the vegetable oil's composition, botanical origin, and quality factors [36].

NIRS can be used to evaluate a number of chemical components and quality factors of vegetable oils. These consist of the content of free fatty acids, moisture content, triglyceride profile, oxidative stability, and the presence of contaminants or adulterants. The purity and authenticity of the vegetable oil can be ascertained by comparing the NIRS data with a calibration model that has already been designed and constructed using real samples with known characteristics. An essential component of NIRS analysis is the creation of trustworthy calibration models [37]. By comparing spectral data with reference data acquired using traditional analytical techniques like gas chromatography (GC) or high-performance liquid chromatography (HPLC), the models are constructed. In order to evaluate the predictive power of the model, the calibration procedure entails choosing suitable spectral regions, preprocessing the data, choosing regression mathematical algorithms, and performing cross-validation. To guarantee the calibration models' accuracy and dependability, they must be updated and validated on a regular basis using a set of reference samples [10]. When it comes to vegetable oil verification, NIRS has many benefits. Its quick analysis makes real-time quality control possible throughout the production, processing, and distribution stages. It minimises the amount of sample preparation needed, which cuts down on sample handling time and expenses. Furthermore, because NIRS is non-destructive, the vegetable oil sample can be reused or subjected to additional examination. Additionally, it works well for examining intricate oil blends or combinations, making it easier to find adulterants or evaluate

the consistency of the product. Nevertheless, NIRS is not without its limitations [4]. To guarantee precise predictions, a comprehensive reference database and a well-built calibration model are necessary. Particle size, moisture content, and homogeneity are examples of sample variables that can interfere with NIRS analysis accuracy and necessitate careful sample preparation and control. Furthermore, the detection and measurement of substances with substantial near-infrared absorbance is the only application for NIRS. To sum up, NIRS is a useful method for vegetable oil authenticity and quality assurance. It is a desirable instrument for routine examination in the food sector due to its speed, ease of use, and affordability. NIRS allows for the detection of adulteration, evaluation of quality characteristics, and identification of the oil's botanical origin by utilising the distinctive infrared spectral signatures of vegetable oils [38].

2.4. High-performance liquid chromatography (HPLC)and contaminantes

Vegetable oil quality control and verification are frequently carried out using high-performance liquid chromatography (HPLC), a potent analytical method. HPLC makes it possible to identify, separate, and quantify certain compounds or groups of compounds found in the oil. It works especially well for analysing non-volatile substances such fatty acids, sterols, pigments, antioxidants, and other trace elements [37]. A liquid mobile phase is used in the HPLC process to move the oil sample through a packed column. A stationary phase in the column interacts with the oil sample's constituents in a targeted manner. Different components are separated as the mobile phase passes through the column according to how well they bind to the stationary phase. Controlling a number of variables, such as the temperature, pressure, flow rate, and composition of the mobile phase, results in the separation [39].

The foundation of HPLC is the idea of differential interaction between the oil's constituents and the stationary phase. A number of variables, including polarity, charge, molecule size, and the existence of particular functional groups, may influence this interaction. Various kinds of columns with certain stationary phases (such as reversed-phase, normal-phase, and ion-exchange) are selected according to the physicochemical characteristics of the analyte and the target molecules. Depending on the kind of analysis, a representative sample of vegetable oils is created by either injecting the oil directly or dissolving it in an appropriate solvent. Following the injection of the sample, it is combined with the mobile phase and run through the column in the HPLC apparatus. Various detectors, such as UV–Vis absorbance detectors, diode array detectors, fluorescence detectors, or mass spectrometry, are typically used to detect the separated chemicals [40].

Vegetable oils can be verified and a variety of parameters can be found using HPLC. Several substances that are frequently examined include: HPLC can separate and quantify individual fatty acids present in the vegetable oil, providing information about the fatty acid composition and profile. This information can be used to differentiate different oil types, authenticate the oil's botanical origin, and detect potential adulteration [25]. Sterols, present in vegetable oils, can be analysed by HPLC to identify specific sterol profiles and determine the oil's authenticity. Sterol analysis can reveal the presence of adulterants or non-authentic sources in the oil sample [37]. HPLC allows for the separation and identification of pigments in vegetable oils, such as carotenoids and chlorophylls. Pigment profiles can vary between different oil sources, providing information about the oil's origin or possible adulteration [29]. HPLC can be employed to identify and quantify natural antioxidants, including tocopherols and phenolic compounds, in vegetable oils. Antioxidant content is an essential quality parameter that can affect the oil's stability, freshness, and nutritional value [2]. The types biomarkers for gastrointestinal cancer is presented in Table 2, and gastrointestinal biomarkers of microbiota with test site and detection methods is presented in Table 3.

Pesticides, mycotoxins, and heavy metals are examples of contaminants or unwanted materials that can be found and measured in vegetable oils using HPLC. These pollutants can be discovered, enabling quality evaluation and guaranteeing consumer safety, by using appropriate detectors and setting analytical methodologies. Vegetable oils can be analysed with flexibility using a variety of detection techniques and column types available in HPLC. This versatility enables the development of specialised procedures to address certain quality characteristics or concerns about adulteration [34]. Calibration curves with established reference standards are created, and sample findings are compared to these standards to guarantee correct results. Furthermore, appropriate sample preparation methods, like extraction or filtration, are used to get rid of any contaminants that can interfere with the analysis. To sum up, HPLC is a flexible method for the examination and verification of vegetable oils. The evaluation of fatty acid profiles, sterol composition, pigment content, antioxidant levels, and the presence of pollutants is made possible by its capacity to separate and quantify specific compounds or classes of compounds. HPLC helps ensure consumer confidence and product integrity by contributing to the authenticity and quality control of vegetable oils by providing information about the oil's composition [1].

Table 2
Types of Biomarkers for gastrointestinal cancer.

Type of biomarker	Example	Gastrointestinal tumor type	References
Histologic staging	Dysplasia and cancer staging	All gastrointestinal cancers	[5]
Genetic and family risk	FAP testing	Colorectal cancer	[9]
Susceptibility	Multiple cytokine polymorphisms	Gastric	[10]
Diagnostic	Carcino-embryonic antigen	Colorectal cancer	[11]
Prognostic	P53, p16	Esophageal adenocarcinoma	[12]
Predictive	ErbB2 for trastuzumab therapy, EGFR for gefitinib therapy	Gastric	[13]
Exposure	Nitrites/DNA adducts	Esophageal adenocarcinoma	[14]

Table 3
Gastrointestinal Biomarkers of microbiota with test site and detection methods.

Biomarker	Test site	Biological sample	Method	Comments	References
Lactate	Whole intestine	Blood, digesta content	Colorimetric Fluorometric	Indirect measurement of intestinal permeability as lactate can go across the intestinal mucosa to blood	[10]
Succinate	Whole intestine	Digesta content	Colorimetric	Phenolic compounds produced by microbial fermentation of aromatic amino acids, these volatile organic compounds could be quantified with electronic noses and other portable sensors	[5]
Phenol	Whole intestine	Blood, urine, faeces	Gas – chromatography, mass spectroscopy, nuclear magnetic resonance	Associated with high levels of dietary proteins, leading to excessive microbial fermentation	[15]
Ammonia	Large intestine	Faeces, urine	Colorimetric	Associated with high levels of dietary proteins, rich in sulphur – containing amino acids, and inorganic sulphur	[16]
Hydrogen sulphide	Large intestine	Faeces	Colorimetric	Associated with high levels of dietary proteins	[17]

2.5. Gas chromatography (GC)

Vegetable oil quality control and authenticity are frequently carried out using gas chromatography (GC). It works particularly well for analysing volatile and semi-volatile substances found in oil. With the use of GC, individual compounds or classes of compounds can be separated, identified, and quantified, yielding important details regarding the composition, legitimacy, and purity of the oil. In a gas chromatograph (GC), which has a separation column, an injector, a carrier gas system, and a detector, the sample is vaporised and injected [23]. Usually, a stationary phase that interacts with the sample components is coated on the separation column. The vaporised sample is transported through the column at a regulated flow rate by the carrier gas, such as nitrogen or helium. Following injection, the components of the sample are separated according to how differently they interact with the stationary phase. Stronger interactions between compounds and the stationary phase will cause slower migration and longer retention durations. On the other hand, substances that exhibit weaker interactions will elute more quickly and have shorter retention periods [29].

After being separated, the individual chemicals are found using an appropriate detector, most frequently a mass spectrometer (MS) or a flame ionisation detector (FID). The capacity of organic compounds to ionise in a hydrogen flame is the basis for the FID's detection of them, whereas the mass-to-charge ratio of the ionised molecules gives the MS additional structural information. Vegetable oils contain a wide range of substances that can be analysed using GC, including hydrocarbons, fatty acids, triglycerides, sterols, tocopherols, volatile fragrance compounds, and other minor components [37]. The authenticity, quality, and purity of the vegetable oil can be evaluated by quantifying these components and comparing them to accepted standards or criteria. Depending on the target substances and the type of analysis, GC can be utilised in a variety of modes, including chiral chromatography, normal phase, and reversed-phase chromatography. The precise needs of the study determine the column to be used as well as the operational circumstances (temperature, carrier gas flow rate, and column dimensions [14].

Calibration curves are usually built using reference standards with known concentrations of compounds to improve accuracy and dependability. The concentration of each component or class of compounds is then ascertained by comparing the sample results with the calibration curves. Additionally, to get rid of interfering molecules or make some compounds easier to detect, sample preparation methods like solvent extraction or derivatization may be used [32]. To sum up, gas chromatography (GC) is a flexible method for vegetable oil verification and quality assurance. The evaluation of important elements and indicators that characterise the content and quality of the oil is made possible by its capacity to separate, identify, and quantify volatile and semi-volatile chemicals. GC analysis supports consumer confidence and safety by helping to ensure the authenticity, purity, and overall integrity of vegetable oils [24].

2.6. Isotope ratio mass spectrometry (IRMS)

Vegetable oil authenticity is accomplished through the use of a specialised technology called isotope ratio mass spectrometry (IRMS). It uses variations in the stable isotope ratios of elements including oxygen (δ18O), hydrogen (δ2H), and carbon (δ13C) to ascertain the origin, location, and legitimacy of the oils. The idea behind Integrated Root Mean Square Distribution (IRMS) stems from the observation that the isotopic composition of plants used to produce vegetable oils varies depending on growth conditions, environmental factors, and photosynthetic routes. Subtle differences exist in the abundances of isotopes of an element with varying neutron counts. A mass spectrometer can be used to quantify and analyse these changes in order to distinguish between different sources of vegetable oils [23].

The oil sample must go through a number of preparation stages in order to apply IRMS for vegetable oil authentication. The intrinsic isotopic fingerprints of the lipids are first preserved during the extraction process. After that, the samples are transformed into gaseous or volatile states so that they may be put into the mass spectrometer for isotope analysis. Fatty acid methyl esters (FAMEs) or other volatile forms that may be examined using gas chromatography-isotope ratio mass spectrometry (GC-IRMS) or liquid chromatography-isotope ratio mass spectrometry (LC-IRMS) are typically produced from the extracted lipids [8]. In GC-IRMS, a mass spectrometer is used to determine the isotope ratios and gas chromatography is used to separate the FAMEs. Non-volatile components in LC-IRMS are separated and isolated using liquid chromatography before being added to the mass spectrometer for isotopic analysis.

The vegetable oil sample's isotopic composition is represented by a δ value, which is the sample's isotopic ratio's divergence from a reference standard. Parts per thousand (‰) is the standard unit of reporting for the δ value. The geographical origin of the oil can be determined or probable adulteration can be detected by comparing the δ values of the oil sample with reference values from reliable sources [27]. Analysis of the carbon isotope ratio (δ13C) is frequently used for vegetable oil verification. Based on the kind of photosynthetic pathway used by the plant during growth, it aids in oil differentiation. Plants that follow the C4 pathway, including maize and sugarcane, have different isotopic compositions than C3 plants, such soybean and olive. The kind of plant the vegetable oil came from can be identified by testing the δ13C readings of the oil. To get further details, other isotopes like oxygen (δ18O) and hydrogen (δ2H) can also be examined. For example, the composition of oxygen isotopes can reveal the location of the water source used by plants during their growth [36]. The climate and environmental elements in the plant's growth zone may be reflected in the hydrogen isotope ratio. When it comes to vegetable oil authenticity, IRMS has many benefits. It offers a straightforward and impartial way to ascertain the oil's provenance and place of origin. The method is extremely sensitive and precise, able to detect even minute variations in isotope ratios. Moreover, IRMS can be used with a variety of oils, regardless of how or what form they are processed. Still, there are a few difficulties with IRMS analysis. Accurately doing isotope analysis calls for specific tools and training. Establishing and maintaining appropriate quality control and calibration protocols is necessary to guarantee the accuracy and dependability of the outcomes. Creating extensive reference databases with isotopic values for real vegetable oil samples from various locations is also crucial [35].

To sum up, Isotope Ratio Mass Spectrometry (IRMS) is an effective method for confirming the provenance and authenticity of vegetable oils. Through the analysis of carbon, hydrogen, and oxygen stable isotope ratios, IRMS offers important insights into the oil's provenance, authenticity, and possible adulteration. By boosting consumer confidence and facilitating efficient quality control procedures, IRMS helps to ensure the integrity and quality of vegetable oils [28].

2.7. Immunological methods

Vegetable oil authenticity has shown to benefit greatly from the application of immunological techniques, commonly referred to as immunoassays. These techniques rely on the application of particular antibodies that have the ability to attach to target molecules or chemicals in the oil sample in a selective manner. Immunological techniques are advantageous for both laboratory and field-based applications due to their high specificity, sensitivity, and ease of use [37]. Various immunological techniques can be applied for the verification of vegetable oil. Below is a description of some of the methods that are frequently used.

2.7.1. Enzyme-linked immunosorbent assay (ELISA)

An extensively utilised immunological technique for examining a variety of analytes, such as particular substances or impurities in vegetable oils, is ELISA. An antibody specific to the target analyte, which is immobilised on a solid support like a microplate, is used in ELISA. If the target analyte is present, it will bind to the immobilised antibody when the vegetable oil sample is added to the well containing the immobilised antibody. Next, a substrate that generates a detectable signal when the enzyme is present is added, along with a secondary antibody that has been conjugated to an enzyme and binds to the target analyte. The signal that emerges is proportionate to the target analyte's concentration in the sample of vegetable oil [10].

2.7.2. Lateral flow devices (LFDs)

LFDs are quick and portable immunological techniques that can be applied for on-site examination. They are sometimes referred to as immunochromatographic assays or strip tests. These straightforward and easy-to-use instruments comprise a membrane strip with certain antibodies immobilised at several test lines along with a control line [37]. The vegetable oil sample is placed to the sample pad and moves along the strip due to capillary action in order to conduct the test. A noticeable coloured band will form on the test line if the target analyte is present in the sample because it will bind to the associated antibody. The control line is used to confirm that the test is operating as intended. A reader's assistance or visual evaluation of the band intensity can yield a semi-quantitative or qualitative outcome [29].

2.7.3. Immunoaffinity chromatography (IAC)

IAC is the process of selectively capturing and concentrating target analytes from a complex mixture, such vegetable oil, using antibodies that have been immobilised on a solid substrate, like a column. After the vegetable oil sample is run through the IAC column, some of the components are washed away and the target analyte binds to the immobilised antibody. After being collected, the analyte can be eluted and subjected to additional analysis or quantification via mass spectrometry or HPLC. The creation and selection of antibodies are essential to the efficacy of immunological techniques. Animal immunisations can yield antibodies, or monoclonal antibodies can be produced by recombinant DNA technology. The appropriateness of antibodies for target analyte detection in vegetable oils is determined by their specificity and affinity [10].

There are various benefits of using immunological techniques for the verification of vegetable oil. Because of their extreme specificity, they may identify target molecules or contaminants even in intricate matrices. These techniques are sensitive enough to pick up analytes even in very small quantities. Furthermore, immunological techniques don't require a lot of sample preparation and are quick and easy to use. Nevertheless, immunological approaches have certain drawbacks. False-positive or false-negative results may arise from matrix interferences or cross-reactivity with related chemicals [4]. Antibody selectivity and specificity should so be thoroughly assessed and verified. The accuracy and dependability of immunological approaches can also be impacted by matrix effects and sample complexity, necessitating appropriate sample preparation and validation techniques. To sum up, immunological

techniques, such ELISA, LFDs, and IAC, offer excellent specificity and sensitivity for the identification of target molecules or contaminants in vegetable oil samples, making them valuable tools for the authenticity of vegetable oils. They can be modified for on-site testing or laboratory-based analysis, and they are comparatively easy to operate. The use of immunological techniques aids in quality control procedures that guarantee the reliability and security of vegetable oil products [36].The different types of biomarkers and their functionality are presented in Fig. (2).

2.8. Nuclear magnetic resonance (NMR) spectroscopy

Vegetable oils are identified and characterised using the potent analytical method known as nuclear magnetic resonance (NMR) spectroscopy. It offers useful details regarding the molecular makeup, composition, and characteristics of the oil, making it possible to determine important constituents, spot adulteration, and evaluate quality standards. The basis of nuclear magnetic resonance, which describes how atomic nuclei interact with a magnetic field, is how NMR spectroscopy operates [37]. A sample having spin-bearing nuclei, like carbon (13C) or hydrogen (1H), will align either with or against the external magnetic field. The nuclei can change from one spin state to another by exposing them to electromagnetic radiation in the form of radiofrequency pulses at a particular energy. To learn more about the sample, the energy absorption or emission that results is found and quantified [10].

Vegetable oil samples are placed in an NMR spectrometer, which produces a high magnetic field and emits radiofrequency pulses, in order to use NMR spectroscopy for vegetable oil verification. The distinct frequencies at which the nuclei in the oil, like 1H or 13C, vibrate are determined by their chemical interactions and surroundings. Valuable molecular information can be gained by measuring the intensities of the frequencies, also known as chemical shifts [10].

The types and amounts of fatty acids found in vegetable oils can be inferred from their NMR spectra. The spectrum can be used to identify and quantify the unique chemical shifts and multiplets that are displayed by various fatty acids. This makes it possible to determine the fatty acid profile and aids in distinguishing between various oils or sources of oils [34].

Vegetable oil rancidity and oxidative degradation indicators can be found using NMR spectroscopy. Spectral patterns or peak intensities can be utilised to identify chemical changes brought on by oxidation, such as the hydroperoxides or triglycerides breaking down. This makes it possible to evaluate the freshness and quality of the oil [24,28].

Vegetable oils can have impurities or adulterants that can be found and identified via NMR spectroscopy. Differences or inconsistencies between the NMR spectra of probable adulterants and authentic samples can be seen, suggesting the possibility of adulteration or non-authentic components [27]. Proteomics as a promising biomarker in food authentication and its functionality is presented in Fig. (3).

It has been demonstrated that NMR spectroscopy may be used to identify the botanical and geographic origins of vegetable oils. Different plant species, growing environments, and environmental factors can produce distinctive NMR spectrum patterns that can be used to identify the source of the oil or potential adulteration [29].

Vegetable oil crystallisation properties and solid fat content can also be examined using NMR spectroscopy. These factors can impact the stability and sensory qualities of vegetable oil products, making them significant for a range of food applications. Proficiency in spectral interpretation, coupled with access to calibration models and reference databases tailored to vegetable oil authenticity, are prerequisites for analysing NMR spectra. For the purpose of authentication, NMR spectra are frequently analysed and

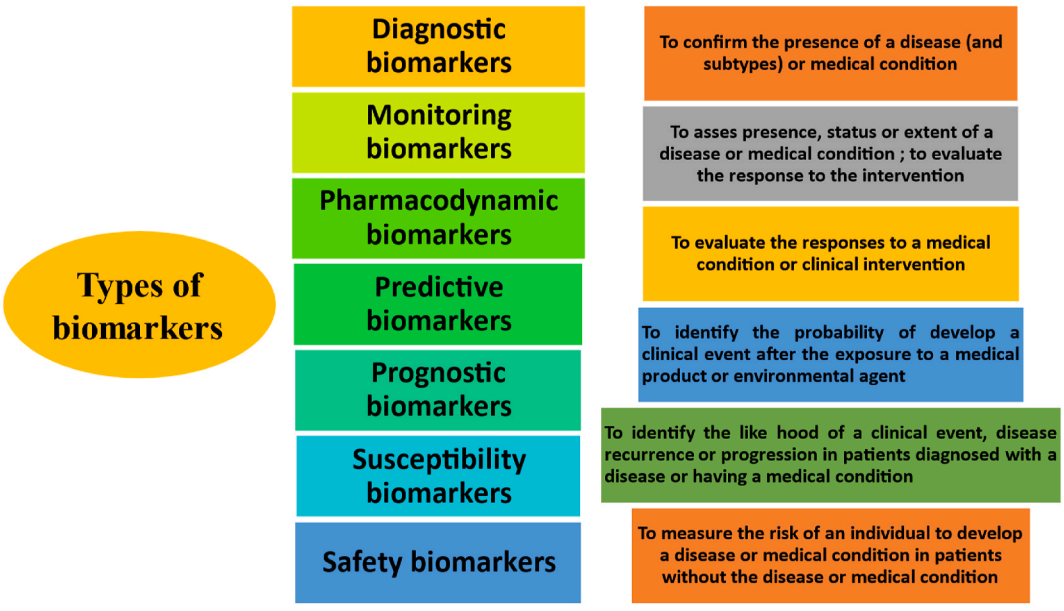

Fig. 2. Types of biomarkers and their functionality.

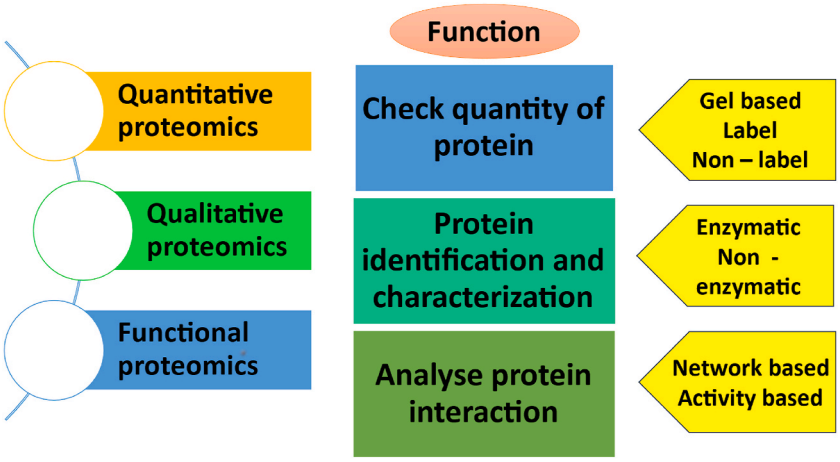

Fig. 3. Proteomics as a promising biomarker in food authentication and its functionality.

compared using sophisticated data processing techniques including chemometric analysis and pattern recognition algorithms [4]. NMR spectroscopy has several benefits when it comes to the verification of vegetable oils. Because it is non-invasive and non-destructive, the oil sample can be reused or subjected to more study. NMR allows for the simultaneous provision of quantitative and qualitative data about several components, facilitating thorough analysis. NMR spectra can also be obtained rather quickly, which makes them appropriate for use in quality control and research applications. Nevertheless, NMR spectroscopy has certain drawbacks. Accurate operation and interpretation of the spectra necessitates specialised equipment and skilled staff [36]. In order to get accurate results, NMR spectrometers can be expensive and may need sample preparation methods like homogenization or solvent extraction. Furthermore, the use of NMR spectroscopy is typically restricted to low analyte concentrations, necessitating the use of concentration procedures for trace or minor components. To sum up, NMR spectroscopy is an effective method for characterising and authenticating vegetable oils. It offers important details regarding the oil's molecular makeup, fatty acid profile, degree of oxidation, and place of origin [28].

2.9. Electrophoresis techniques

Vegetable oil authentication is a common use for electrophoresis methods including gel electrophoresis and capillary electrophoresis (CE). These methods are predicated on the analysis and separation of molecules according to their size, charge, or other physical characteristics. Vegetable oil authentication can benefit from electrophoresis techniques in a number of ways, including high resolution, adaptability, and simultaneous analysis of several components [35].

2.9.1. Capillary electrophoresis (CE)

A potent method for separating charged molecules according to their electrophoretic mobility in an electric field is capillary electrophoresis. The separation column in CE is a capillary that is filled with a buffer solution. When the sample is inserted into the capillary, an electric field is generated, which causes the charged molecules to move according to their charge-to-size ratio through the capillary. It is possible to separate and quantify different molecules because of how they move in the electric field. Vegetable oils contain a variety of constituents that can be analysed using CE, including as phenolic compounds, triglycerides, sterols, antioxidants, and fatty acids. Assessing the authenticity, quality, and possibility of adulteration of vegetable oil samples can be done by comparing their electrophoretic profiles with those of unknown samples [34].

2.9.2. Gel electrophoresis

Another popular electrophoretic method for analysing compounds in vegetable oils is gel electrophoresis. With this technique, molecules are separated according to size using an electric field and a gel matrix, such as agarose or polyacrylamide gel. Depending on the particular needs of the analysis, there are various ways to do gel electrophoresis, such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and polyacrylamide gel electrophoresis (PAGE). Proteins in vegetable oils can be separated and identified using gel electrophoresis. Applying the oil sample to the gel and then electrophoresizing it will yield protein profiles [10]. It is possible to identify certain protein markers that are indicative of various vegetable oil variations or adulterants by comparing protein profiles with established references. Electrophoresis techniques can be used to separate and identify components as well as offer extra information by combining them with other detection methods. To see the separated components, gel electrophoresis can be followed by staining techniques like silver or Coomassie Brilliant Blue. To improve sensitivity and detect specific targets, electrophoresis can also be used in conjunction with fluorescent or chemiluminescent detection techniques [34].

Standardised procedures and suitable reference materials must be developed in order to guarantee the precision and dependability of electrophoresis-based methods for vegetable oil authenticity. An extensive collection of vegetable oil samples with well-established

features found in reference databases are essential for accurate electrophoretic profile analysis and interpretation. There are various benefits to using electrophoresis techniques for the authentication of vegetable oil. They make it possible to evaluate several elements at once and offer thorough details about the composition of the oil [31]. Comparing electrophoresis procedures to other analytical techniques, they are also more affordable and straightforward. When employing electrophoresis for vegetable oil verification, there are a few restrictions to take into account. The selection of separation parameters, such as pH, voltage, temperature, and buffer composition, has a significant impact on the analysis. To get precise and consistent results, these conditions must be optimised. Furthermore, the resolution and interpretation of electrophoretic profiles might be impacted by matrix effects and sample preparation, necessitating the use of suitable sample preparation methods and validation processes [35]. To sum up, electrophoresis methods like gel and capillary electrophoresis offer useful instruments for vegetable oil quality assurance and certification. These techniques help determine the validity and quality of oil by enabling the separation and identification of constituents including proteins, fatty acids, and other charged compounds. Techniques for electrophoresis provide efficient quality control measures for vegetable oil products and work in tandem with other analytical approaches [36].

2.10. Electronic Nose (E-nose) and Electronic Tongue (E-tongue)

Emerging analytical methods for vegetable oil quality monitoring and authenticity include Electronic Tongue (E-Tongue) and Electronic Nose (E-Nose). These methods, which are based on arrays of sensors that replicate human taste and smell receptors, allow for the recognition and distinction of intricate flavour and odour profiles connected to various vegetable oil types or adulterants.

2.10.1. Electronic Nose (E-nose)

An E-Nose is a device that analyses and finds the volatile organic compounds (VOCs) released by a sample using a variety of non-specific gas sensors. These sensors react differently to many kinds of molecules according on their size, charge, and other physicochemical characteristics. A distinctive "fingerprint" of the sample is subsequently produced by applying multivariate analytic techniques to the pattern of sensor responses. These fingerprints can be used to identify adulterants in the oil or to authenticate it by comparing them to reference profiles from known vegetable oil samples. E-noses have proven to be an effective tool for analysing volatile substances found in vegetable oils, such as oxidation products, volatile pollutants, and fragrance molecules. They can offer details regarding the oil's purity, freshness, and possible adulteration. E-Noses are appropriate for industrial applications since they can quickly and non-destructively analyse large batches of samples [36].

2.10.2. Electronic Tongue (E-tongue)

With a variety of sensors that react to various aspects of taste, an E-Tongue is a gadget that simulates human gustatory perception. Certain ions, pH, electrical conductivity, and other physicochemical characteristics linked to taste can all be detected by these sensors. The sample's taste profile is then produced by combining the sensor responses. To evaluate the flavour profiles of vegetable oil samples and find differences or adulteration, multivariate analysis techniques are used [38].

E-tongues have been utilised to evaluate the bitter, sour, sweet, and astringent tastes of vegetable oils. They can offer details regarding the oil's sensory qualities, legitimacy, and possible adulteration. E-Tongues, akin to E-Noses, provide quick and non-invasive analysis, making them valuable for extensive quality control in the food sector. For the purpose of authenticating vegetable oil, both E-Noses and E-Tongues have various benefits. Their quick, impartial, and objective analysis lessens the differences in human perception. These methods enable high-throughput analysis by analysing multiple samples at once. Additionally portable and appropriate for on-site testing, E-Noses and E-Tongues provide for real-time quality monitoring at various manufacturing or distribution phases [4]. There are a few restrictions to take into account, though. E-Noses and E-Tongues are limited in their ability to offer extensive chemical information since they analyse general odours or taste profiles rather than particular chemicals. Furthermore, environmental variables like temperature, humidity, and sample matrix might affect the sensors' performance, therefore accurate calibration and validation are required. Vegetable oil profile reference libraries or databases are necessary for precise authenticity and adulteration detection. In conclusion, promising methods for the authentication and quality control of vegetable oils are provided by Electronic Tongue (E-Tongue) and Electronic Nose (E-Nose) procedures. These methods can reveal details about the oil's quality, authenticity, and possible adulteration by analysing taste or odour profiles. In the vegetable oil sector, e-noses and e-tongues provide effective quality control procedures, increased consumer confidence, and improved product integrity [31].

2.11. Microscopy techniques

Microscopy, particularly optical microscopy and microscopy in conjunction with cutting-edge imaging methods, is an invaluable instrument for vegetable oil quality assurance and verification. It makes it possible to visually inspect and characterise a variety of tiny elements, such as adulterants, pollutants, or cellular structures, which can reveal crucial information about the oil's quality, validity, and botanical provenance.

2.11.1. Optical microscopy

Using visible light and magnifying lenses, optical microscopy allows for the observation and analysis of samples. It offers the capacity to view the overall structure and look of vegetable oil samples, including their colour, transparency, and particle or sediment content. Additionally, the presence of organic and inorganic impurities, such as dust, foreign materials, or pieces of insects, can be detected by optical microscopy and may be a sign of incorrect processing or adulteration. A specialised method called polarised light

microscopy (PLM) can be used to investigate materials that are birefringent, including crystals or specific fat crystals found in vegetable oils. The distinctive Maltese crosses or interference patterns exhibited by these materials can be seen by PLM employing polarizers and analyzers, which helps determine the oil's botanical provenance or processing parameters [37].

2.11.2. Microscopy combined with imaging techniques

Cutting-edge microscopy methods, like confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), can reveal specific details regarding the composition and microstructural characteristics of vegetable oils. High-resolution imaging of the oil's surface structure is made possible by SEM, which makes it possible to identify and characterise different types of particles, pollutants, or crystal formations. Additionally, it can offer details regarding the morphological characteristics of the oil, including droplet size, aggregation, and emulsion presence [4]. Through the use of laser scanning technology and fluorescence microscopy, CLSM produces high-resolution optical sectioning images of samples. It can be used to see particular elements or parts of the oil, including lipid granules or pigments, giving information on quality standards and potential adulteration. Combining microscopy techniques with certain probes or staining techniques can improve the visibility and identification of vegetable oil constituents [10]. For example, certain proteins, lipid droplets, or other particular substances present in the oil can be identified and distinguished using certain staining methods or fluorescence-based probes. The use of microscopy techniques in vegetable oil verification has many benefits. They offer clear visual data, making it possible to examine both large and small characteristics that can reveal information about the oil's quality, validity, or adulterant content. These methods are widely accessible, reasonably easy to use, and suitable for a variety of sample sizes and types. When employing microscopy for the verification of vegetable oil, there are a few restrictions to take into account [36]. The qualitative character of microscopy techniques necessitates the use of skilled analysts for proper interpretation; factors that may hinder the observation and identification of microscopic features include the complexity of the sample, the presence of substances that interfere with the image, or the long sample preparation required. In conclusion, microscopy methods—such as optical microscopy and microscopy in conjunction with cutting-edge imaging technologies—offer important new information for vegetable oil quality assurance and verification. These methods help with the evaluation of quality criteria, the identification of contaminants, and the detection of adulterants by enabling the visualisation and characterisation of both macroscopic and microscopic aspects. Microscopy enhances consumer confidence and supports efficient quality control methods by contributing to the overall integrity and safety assessment of vegetable oil products [8,40].

3. Combination techniques for vegetable oil authentication

3.1. DNA based method that target nuclear or micocondrial marker

The intricacy of fraudulent techniques makes it difficult to conduct studies on the authenticity and traceability of extra virgin olive oil. Numerous chemical and biological procedures have been established to ascertain the authenticity of olive oil; however, due to their high specificity, sensitivity, and dependability, non-conventional methods based on DNA analysis have garnered interest in recent years. Since genetics plays a major role in determining a species' distinctive identity, DNA analyses have a very high discriminating capacity [41]. Genetic variations in populations or species are referred to as polymorphisms. Molecular markers are useful techniques for identifying the authenticity of olive oil since they offer information on genetic differences. Since the variety or plant species from which the oil was extracted can be identified by the application of genetic markers in the study of the remaining oil DNA, a number of DNA-based methods for authenticating olive oil have recently been developed [15].

Due to their excellent specificity, sensitivity, and accuracy in identifying the botanical origin of plant oils as well as the varietal origin of vegetable oils, unconventional techniques based on DNA analysis have drawn attention [42]. Since DNA-based approaches only rely on the analysis of DNA, they provide an alternative, complementary approach that overcomes the shortcomings of conventional methods, such as the denaturation of proteins. Numerous biomolecular techniques have been developed to use DNA markers for vegetable oil authenticity. Molecular markers are thought to be useful instruments for establishing the authenticity of vegetable oil since they offer details on polymorphisms within DNA regions. Genetic differences known as polymorphisms can be found in the genomes of other organelles, such as chloroplasts, as well as in the nuclear, ribosomal, or mitochondrial genomes.

Since analysis of the residual oil DNA using molecular markers can identify the variety or the species from which it was extracted, regardless of the environmental conditions during olive fruit's growth, several techniques based on DNA have been developed to authenticate vegetable oil. Numerous research used molecular markers, such as SNPs, to identify the varietal origin of vegetable oil [34]. The database of olive molecular markers was enhanced and the analytical targets expanded with the latest developments in vegetable oil genome and transcript sequencing. By focusing on analytes that can be species-specific in terms of nucleotide sequence and DNA length, DNA-based techniques can detect if vegetable oil has been tampered with to make oil from another plant. Analysing species-specific DNA fragments or polymorphisms, or the genetic differences between or within species, is a common procedure in DNA-based analytical methods. The ability to distinguish between very unrelated creatures, as in the cases of food allergies and genetically modified organisms (GMOs), is a feature of species-specific DNA fragments. The ability to distinguish between closely related species or varieties using molecular markers is a feature of polymorphism detection [12].

Using universal primers, the desired DNA target is amplified in a PCR assay for food verification purposes, producing an amplified DNA fragment that can be measured for length. Every plant species has a species-specific PCR product length because of insertions or deletions. The number of PCR products produced for food mixes including many species will match the number of species in the mixture. Thus, the identification of the mixture including species would result from the length of the PCR product being analysed using conventional gel electrophoresis [23]. More advanced analytical tools can be utilised for validation, or if a better resolution is required

because of the possibility of nearly identical length amplicons [40].

3.2. DNA metabarcoding for vegetable oil authentication

Seafood species authentication has made extensive use of DNA barcoding that targets the cytochrome *c* oxidase subunit I (COI) gene, a relatively conserved area with sufficient diversity among species [17]. An developing method that gets beyond the limitations of Sanger sequencing is DNA metabarcoding, which combines DNA barcoding using next-generation sequencing (NGS) to detect numerous species in complex and processed meals. Using pieces as tiny as 150 bp of mitochondrial 16S rDNA, bivalve species from the Mytilidae (mussels), Pectinidae (scallops), and Ostreidae (oysters) families were effectively identified in food items by DNA metabarcoding [11]. An examination of mammalian and poultry species found in food and pet food also proved that DNA metabarcoding employing 16S rDNA is feasible.

Short, consistent DNA sequences are used to distinguish across species, which is the basis for the use of DNA barcoding in food verification. Targeting certain genomic regions, like mitochondrial DNA (mtDNA) or chloroplast DNA (cpDNA), which show enough variation between species while preserving conserved areas within the same species, is the goal of the technique [5]. The capacity of DNA barcoding to identify adulteration and substitution in intricate food matrices is one of its main benefits [21]. Even in processed or highly fragmented food, the approach can distinguish between closely related species or identify the presence of non-declared substances. For example, DNA barcoding can reveal fraudulent activities by detecting the real species present in the sample when premium and costly vegetable oil species are exchanged with less expensive ones. In a similar vein, it can identify allergens that could endanger customers' health. Moreover, DNA barcoding can help identify certain cultivars or geographic origins, offering important details on product quality, cultural heritage, and adherence to geographical indication laws [43]. Global interest in the application of DNA barcoding to stop food fraud has grown significantly. Stakeholders in the industry, governments, and regulatory bodies understand its potential to uphold market integrity, safeguard consumer rights, and guarantee food authenticity. A number of nations and international organisations have launched campaigns and enacted laws in an effort to encourage the use of DNA barcoding as a common method for food verification in recent years. These include the International Organisation for Standardisation (ISO) guidelines on DNA-based technologies for food authenticity testing, the EU Agri-Food Fraud Network (FFN), and the US Food and Drug Administration's (FDA) GenomeTrakr programme. DNA barcoding has several advantages, but it is not without drawbacks. For broader adoption and successful implementation, issues with sample preparation, DNA extraction, database completeness, and the availability of appropriate reference materials must be resolved. Furthermore, to improve the precision, effectiveness, and dependability of DNA barcoding in food fraud detection, continual developments in DNA sequencing technology, bioinformatics tools, and reference databases are essential.

3.3. Real time PCR technique for vegetable oil authentication

It is possible that the presence of PCR inhibitors will significantly reduce the amount of amplifiable DNA fragments that can be obtained. The tiny amount of retrieved DNA presents another challenge in the particular situation of vegetable oils. Using hydrolyzed fluorescent probes, real-time PCR was used to amp up all of the extracts in order to validate the qualitative PCR results and obtain an estimate of the amount of DNA. The ISO 21570-recommended primers and probes were used for the assays. Utilising the oligonucleotide primers Lectin-F/Lectin-R and probe Lectin-TMP, the lectin gene was employed as the reference gene. Target DNA PCR amplicons can be sequenced or subjected to high-resolution capillary electrophoresis or agarose gel electrophoresis analysis. The detection limit of capillary electrophoresis for DNA fragments is substantially lower. Compared to agarose gel electrophoresis, it is more automated and capable of differentiating DNA sequences that differ by a small number of nucleotides in length [6,44]. Quantitative real-time PCR and high-resolution melting analysis (HRM) are two methods that are becoming more and more popular because they provide the benefit of simultaneous PCR amplification and genotyping. Another method is Next-Generation Sequencing (NGS), which lowers the cost of analysis and reveals DNA fragments from multiple species at the same time by providing massive amounts of parallel short-read data per run [13].

3.4. Taqman real time PCR assay

A very sensitive and fast quantitative analysis technique is urgently needed for the timely detection of tainted and inferior oils in vegetable oils. Because of its distinct and priceless flavour, beef tallow was widely used in baked goods and hot pot flavouring. The TaqMan real-time quantitative polymerase chain reaction method was developed for the cytochrome *b* gene (Cyt *b*) in mitochondrial DNA to identify beef tallow quantitatively [19]. As little as 0.1 % and 0.004 ng of beef tallow DNA, respectively, can be found in tallow mixtures using the TaqMan qPCR. A normalised ΔCq technique based on Cyt *b* and 18S rDNA was developed for the quantitative analysis of oils, and it was very accurate and precise when tested on simulated oil samples.

Real-time fluorescence quantified polymerase chain reactions (RT-PCR) studies on the detection of vegetable oils are becoming more and more prevalent both domestically and internationally. The shape of the amplification and the technique of DNA extraction are crucial factors in the successful identification of vegetable oils using qPCR. Vegetable oil was identified using high resolution melting (HRM) based on RT-PCR, showing that HRM is a practical and affordable way to find adulterated vegetable oil.

The dye technique (using dyes like SYBR Green I and Eva Green) and the probe-based method (using probes like TaqMan probe) are the two main RT-PCR procedures. With the dye method, RT-PCR has a relatively high detection limit and a low specificity. Target DNA molecules can be amplified with specificity, efficiency, and sensitivity using the TaqMan real-time PCR (TaqMan qPCR)-based probe

[45]. The TaqMan qPCR quantification approach, which is based on the normalised ΔCq, has been widely utilised to determine and measure meat items and dairy products due to its high accuracy and low deviation [46]. It is anticipated that this method will be utilised for the quantitative identification of beef tallow.

3.5. Genetic sequencing

The creation of trustworthy and precise techniques for vegetable oil verification is required by the numerous instances of adulteration and fraud. In order to genetically identify various species, subspecies, or cultivars, DNA-based techniques examine the remaining DNA that was extracted from vegetable oil and employ molecular markers, which function as indicators that represent unique genetic profiles. The most recent markers for vegetable oil authenticity, such as single nucleotide polymorphisms (SNPs) and microsatellites or Simple Sequence Repeats (SSR), are taken from the DNA and examined. The article also discusses other analysis techniques like qPCR and digital PCR, with a focus on the High-Resolution Melting (HRM) post-polymerase chain reaction method. HRM allows for the fast and accurate identification of genetic variants in DNA regions of interest without the need for sequencing, and it can distinguish between cultivars that are very similar but differ by a single nucleotide in a particular locus. Genetic or molecular markers, which are distinct DNA sequences with known physical locations on chromosomes and can be used to identify individuals or species, are sequences of DNA that are unique to each organism. "Simple sequence repeats" and "single nucleotide polymorphisms" are two frequent genetic markers that have been employed for the identification of olive cultivars and the traceability of vegetable oil [47]. When examining genetic links in olive trees, RAPD, AFLP, and ISSR are advantageous primarily because the PCR primer building process does not necessitate prior knowledge of the genome sequence. The most recent research have employed single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) as genetic markers for vegetable oil verification [48].

An growing number of papers have addressed the appropriateness of DNA markers in giving unambiguous identification for food verification and traceability. Molecular markers have been used as diagnostic tools even in complicated dietary matrices like vegetable oils. Recent developments have led to the specific identification of many plant oils as possible adulterants of olive oil. Further advancements in DNA analysis have been spurred by worries regarding the presence of genetically modified organisms in oilseed crops, which are the source of vegetable oil production. With relation to olive oil specifically, the application of genetic markers has given rise to analytical instruments that evaluate the veracity of cultivar identification as separate indicators from variations in the environment [49].

3.6. Proteomics

Vegetable oil authenticity is crucial for business and health reasons alike. In order to distinguish between cooking oil and edible oil, a new technique known as matrix-assisted laser desorption/ionisation imaging mass spectrometry (MALDI-MSI) was created. With this technology, oil authenticity might be quickly determined by eye inspection without the need for intricate computational analysis. Using a scan range of m/z 280–1860, vegetable oil was analysed in positive ionisation mode. Several diagnostic ions that may be used to identify various kinds of edible oil were found. For the quantification of the main vegetable oil components, such as triglycerides, diglycerides, and monoglycerides, the approach demonstrated good analytical performance [50]. Vegetable oil's chemical composition has been characterised using a range of analytical techniques. Low-polarity oil components, such as volatiles, monoglycerides, fatty acid esters, cyclic, and epoxy compounds, were frequently measured using gas chromatography (GC) coupled to mass spectrometry [51]. In recent years, matrix-assisted laser desorption/ionisation mass spectrometry (MALDI MS) has been developed as a helpful tool to get around some of the drawbacks of these traditional methods. It allows for the quick detection of oil samples without the time-consuming sample pre-treatment and chromatographic separation, as well as the characterization of a wide range of oil ingredients with a diversity of structural characteristics and polarity.

3.7. Metabolomics

Many different kinds of naturally occurring chemicals are produced by edible plants and are essential to their growth and development. Primary metabolites, secondary metabolites, and hormones are the three categories into which low molecular weight phytochemicals are divided [52]. Their presence and concentration are essential for the proper development of plants. The systematic investigation of metabolites and their interactions is made possible by metabolomics. The metabolomics approach was discovered to be a crucial method for food verification more recently [53]. Hazelnut oil might be identified in virgin olive oils by using indicators such as filbertone and 4, 40-dimethylsterols. For the purpose of identifying sunflower or soybean oil in olive oil, a marker known as delta 7-stigmastenol was employed [20].

3.8. Combined techniques of Raman, near-infrared-infrared (NIR) and fluorescence spectroscopy

Edible vegetable blend oils are made up of two or more vegetable oils and are commonly used as a source of dietary fats. Because distinct vegetable oils have different nutritional qualities, blended oils provide a more comprehensive nutrient profile than single vegetable oils. Raman spectroscopy is a method that shows great promise because it can enable non-contact detection and does away with the necessity for sample pre-treatment and other special preparation techniques [7]. Surface enhanced Raman spectroscopy (SERS) is a potent vibrational spectroscopy method that amplifies electromagnetic fields produced by stimulating localised surface plasmons to enable highly sensitive structural identification of low concentration analytes. an innovative liquid interfacial plasmonic

platform that enables quick self-assembly and self-healing, enabling liquid-phase SERS investigation of both water- and oil-soluble compounds that is both highly sensitive and quantitative. In order to achieve the automated classification and identification of naturally and artificially dyed edible bird's nests, two-dimensional correlation surface-enhanced Raman spectroscopy (2DC-SERS) was utilised for the quick quantitative detection of nitrite and nitrosamine in both natural and artificially dyed edible bird's nest samples [16]. As a result, the advancement of Raman spectroscopy for the quick and precise identification of vegetable blended oils may have a big impact on supporting consumer wellness and guaranteeing the veracity of labelling.

NIR spectroscopy is a quick, non-invasive, and sensitive method that operates in the 12,500–4000 cm−1 wavenumber range. It has been extensively used for both qualitative and quantitative investigation of food and agricultural goods. Hydrogen-containing groups in organic molecules, such as O–H, N–H, C–H, and S–H chemical bonds, are the primary targets of NIR spectroscopy. NIR spectra are difficult to understand due to the wide, frequently overlapping bands brought on by combination vibrations and molecular overtones. Therefore, in order to obtain meaningful chemical information, chemometric approaches are required. The NIR spectra of complex substances are challenging to employ directly for quantitative investigation because of the poor absorption and spectral overlap. Typically, chemometrics are used to create a calibration model that includes pertinent relationships between the chemical contents of samples and the overlapping spectra. Common multivariate calibration techniques like partial least squares (PLS), support vector regression (SVR), artificial neural networks (ANN), and extreme learning machines (ELM) have been used extensively in conjunction with NIR spectroscopy as a viable and efficient substitute for adulteration detection or quantitative analysis of complex samples in a variety of industries, including food, medicine, agriculture, and particularly the vegetable oil sector [18].

The benefits of fluorescence (FS) spectroscopy include sensitive, practical, and effective detection. One type of cold luminescence in photoluminescence is called "fluorescence." It functions according to the idea that excited atoms or molecules revert to their ground state when a material absorbs electromagnetic energy. Radiation from electromagnetic sources is the energy emitted during this shift in energy from a higher to a lower energy level. The FS spectrum is the relationship between the FS energy and the appropriate wavelength. A substance's composition can be ascertained based on its FS intensity [9]. When examining oil blends, the FS spectra can overlap since edible oils contain a variety of common fluorophores. To enhance prediction performance, chemometric methods must once more be used to extract and optimise the FS spectra [40]. FS spectroscopy, which has a lower detection limit than other spectroscopic methods, is an effective instrument for measuring individual components of oil in blends of edible oils. PLS in conjunction with synchronous fluorescence spectroscopy (SyFS) was used to measure the amount of vegetable oils in Eucommia ulmoides seed oil. To create quantitative models, they chose the 300–500 nm excitation spectral range. The vegetable oils had LODs as low as 0.48 %. In order to quickly gather extensive FS data, an excitation-emission matrix (EEM) was gathered. The Quasi-Monte Carlo (QMC) integral was then used to calculate the three oils' concentrations and recovery rates. For the trace study of edible oil blends, the low LOD of FS spectroscopy was crucial, as it was in previous investigations employing this analytical method.

3.9. Chromatographic techniques with mass spectrometry

For many years, gas chromatography (GC) has been a reliable method for analysing a wide range of vegetable oil constituents. However, coelution of components in one-dimensional (1D) GC analysis can be problematic as it might restrict accurate identification and quantification of individual analytes. Using multidimensional gas chromatography (MDGC), the sample is separated in two or more independent processes. By raising peak capacity, ideally resolving coelution issues, eliminating underlying matrix or interfering compounds, and identifying compounds present at low abundance by cryofocusing enhancement, the approach can improve separation performance [54]. Comprehensive two-dimensional gas chromatography (GCGC) and traditional heart-cut (H/C) MDGC are two variations of MDGC. The peak's height is increased by a compression zone formed between the two dimensions by a cryogenic modulator [55].

3.10. Gas chromatographic and high performance liquid chromatography

GC and the mass spectrometry (MS) detector are frequently combined. This detector's main benefit is that it makes it possible to identify substances using mass-to-charge (m/z) ratios and the relative abundances of molecular and fragment ions that result from electron ionisation (EI), which typically occurs at 70 eV [56]. MS makes it possible to analyse each component that came out of the gas chromatograph independently. Each component could be identified with certainty thanks to the mass spectra and chromatographic peaks. The mass spectrum for each peak in an unknown combination can help to narrow down the potential identities of each component. Therefore, if there is no supporting identification, the compound identification that is provided by evaluating the MS spectrum and comparing it with a commercial MS library like NIST or Wiley is only regarded provisional.

Vegetable oil authenticity has been constantly changing to fit circumstances that were essentially dictated by an international market trend. Analytical methods have been created or adjusted to provide tenable answers to the cunning adulterations that occur every now and then. Newer technical approaches, primarily based on high-performance liquid chromatography, have essentially supplanted classical tests [57]. The technique known combine is a popular and commonly used method for separating and identifying the constituents of a mixture. This method can also be used to identify lipid peroxides with varying molecular weights, polarities, and/or volatility. The methods most commonly employed to identify contamination and adulteration have been the determination of *trans*-fatty acid and sterolic content, in conjunction with sterol-dehydration products. Complex new adulterations, such as mixing hazelnut oil with olive oil, will be a difficulty in the new millennium, but databases and mathematical algorithms are helping to generate promising suggestions for their detection. Fatty acids, triglycerols, sterols, tocopherols, and hydrocarbons have all been quantified using high performance liquid chromatography (HPLC) analyses.

Table 4
Characteristics of detection techniques, types of biomarkers identified, and research advancements.

Detection Technique	Characteristics	Types of Biomarkers Identified	Research Advancements	References
Gas Chromatography	High sensitivity Separation of compounds Quantitative analysis	Fatty acid composition Sterol profiles Triglyceride composition	Improved resolution and detection limits Development of hyphenated techniques Automated data processing algorithms Integration with mass spectrometry	[18]
Mass Spectrometry	High specificity Identification of compounds Molecular weight analysis	Phytosterol content Oxidation products Alkyl esters	Enhanced ionisation techniques High-throughput methodologies Miniaturization of instruments Advances in tandem MS for complex mixtures	[19]
Nuclear Magnetic Resonance (NMR)	Structural elucidation Quantitative analysis Non-destructive Minimal sample preparation	Triacylglycerol composition Free fatty acids Aromatic compound profiles Lipid oxidation markers	Higher magnetic field strengths Development of benchtop NMR instruments Automation of data interpretation Integration with chemometrics Utilization of heteronuclear experiments	[20] [19]
Metabolomics	Comprehensive analysis of metabolites Profiling of small molecules Dynamic assessment of metabolism	Metabolic pathways Biomarker discovery Disease biomarkers	Enhanced data processing algorithms Integration with multi-omics approaches Standardization of analytical workflows	[21]
Proteomics	Identification of proteins Characterization of protein-protein interactions	Protein expression levels Post-translational modifications (PTMs) Biomarkers for disease diagnosis	Improved protein quantification techniques Enhanced bioinformatics tools for data analysis Integration with other omics data for systems biology studies	[22]

Some biomarkers are well recognized and applied in the authentication of vegetable oils to ensure their purity and safety. Owing to their antioxidant qualities, phenolic compounds are important indicators of the oxidative stability and health-promoting qualities of vegetable oils. Essential biomarkers that indicate the content and purity of vegetable oils are glycerol esters, which are created during the esterification process in oil production. Capsaicin, a bioactive molecule present in chili peppers, is another significant biomarker exploited in the authenticity of oils generated from chili peppers or containing chili extracts. The various characteristics of detection techniques, and types of biomarkers identified are presented in Table 4.

4. Limitation and future scope

Future implications of the discovery and use of novel biomarkers for vegetable oil verification are substantial. To increase the variety of biomarkers and boost their sensitivity and specificity, more investigation is required. This involves looking at cutting-edge tools like genomics, proteomics, and metabolomics in an effort to find more biomarkers that can help verify vegetable oils. Furthermore, the development of artificial intelligence (AI) algorithms and improvements in data analysis techniques will facilitate the standardisation and automation of biomarker-based authentication procedures. Large-scale screening of vegetable oil sample will be facilitated by this, allowing for faster and more accurate analysis. Furthermore, combining several biomarkers and methodologies into multi-dimensional approaches may improve the precision and dependability of vegetable oil authenticity. A comprehensive and holistic approach can be obtained by combining multiple biomarkers, including fatty acids, volatile chemicals, stable isotopes, and DNA markers, thereby reinforcing the authenticity assessment of vegetable oils. In conclusion, the field of finding novel biomarkers for vegetable oil authentication is lively and has a lot of promise. Biomarker-based authentication techniques will be used more frequently as science and technology develop, offering a strong and dependable way to guarantee the safety, authenticity, and quality of vegetable oil products.

5. Conclusion

Rapid advancements in food safety, product integrity, and stopping dishonest practices in the vegetable oil business can be achieved through the development of novel biomarkers for the authentication of vegetable oils. By employing sophisticated analytical techniques like mass spectrometry, NMR spectroscopy, chromatography, and DNA-based approaches, scientists have made significant strides towards identifying particular biomarkers that are capable of differentiating between various vegetable oil varieties, identifying their origin, and spotting adulteration. To guarantee their dependability and practicality, strong biomarker-based authentication techniques must be developed and validated. Accurate biomarker identification and quantification need the establishment of standardised techniques, reference databases, and calibration models. Additionally, in order to put these strategies into practice and enforce quality control standards throughout the vegetable oil supply chain, cooperation between researchers, regulatory agencies, and industry stakeholders is essential.

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Ethical statement - studies in humans and animals

The authors state that this research does not entail using either humans or animals.

CRediT authorship contribution statement

Shivangi Srivastava: Writing – review & editing, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Vinay Kumar Pandey:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kunal Singh:** Visualization, Validation, Software, Formal analysis. **Aamir Hussain Dar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kshirod Kumar Dash:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rafeeya Shams:** Writing – review & editing, Visualization, Validation, Supervision, Software, Formal analysis, Data curation. **Ayaz Mukarram Shaikh:** Visualization, Validation, Supervision, Resources, Funding acquisition, Formal analysis. **Béla Kovács:** Validation, Software, Resources, Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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