

Review Article

Profiling of Plant Derived Natural Constituents by Using Magnetic Resonance Techniques

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Plants are reservoirs of naturally occurring chemical constituents with a wide range of structural diversity. These biological compounds can be derived from different parts of plants such as leaves, barks, seeds, seed coats, flowers, and roots. A broad array of secondary metabolic compounds is present in the plants such as antibiotics, alkaloids, antimicrobials, food-grade pigments, and phenolics which have been reported to possess numerous health-related benefits, including antioxidant, anti-inflammatory, anticancer, and antiobesity activities. Therefore, the identification and detection of these compounds are of utmost importance in order to utilise their benefits into various fields. Wherein, magnetic resonance techniques, such as NMR (nuclear magnetic resonance), MRI (magnetic resonance imaging), and EPR (electron paramagnetic resonance), being far more reproducible, nondestructive, than other analytical techniques such as liquid chromatography, mass spectroscopy, and high-performance liquid chromatography cover a much wider dynamic range of metabolites with easy sample preparation techniques with high speed and fidelity. Hence, these magnetic resonance techniques have been proven to be extremely useful in plant metabolite profiling and disease metabolomics, along with structural elucidation of bioactive compounds from plant sources. Therefore, the present review focuses on the effectiveness of magnetic resonance for the detection of plant-derived metabolites that may lead to new areas of research in various fields such as drug discovery and development, metabolomics, combinatorial chemistry, and assessing overall food safety and quality.

1. Introduction

Plants are a rich source of metabolites that are used in medicines, food additives, nutraceuticals, flavourings, and other commercial applications. In the recent times, there is a high commercial relevance of these secondary metabolites due to their uncountable health benefits which have accounted for a lot of awaking interest to improve their

production in various applications [1]. The small molecules, intermediates, and products produced during metabolism are known as metabolites. Metabolites serve a variety of purposes including fuel, structure, signalling, enzyme stimulation and inhibition, catalytic activity (typically as a cofactor), defence, and ingestion. These metabolites are generally of two types, i.e., primary metabolites such as carbohydrates, vitamins, hormones, and lactic acid which

have a direct role in proper development, growth, and reproduction others are secondary metabolites which have a wide range of active compounds with high biological activity and are biosynthetically derived from primary metabolites only. Flavonoids, steroids, pigments, and alkaloids are the four major chemical families which belong to secondary metabolites [2]. Plant-derived metabolites perform a variety of roles in stress responses including regulating osmotic pressure within cells, preventing oxidation of cell components, and preventing infection and development of pathogenic microorganisms [3]. These metabolites possess various therapeutic properties such as anti-inflammatory, anticancerous, and antidiabetic, which help in the prevention of various diseases such as cardiovascular, respiratory, gastrointestinal, and neurodegenerative disorders caused due to high oxidative stress. Moreover, plant-based foods including vegetables, fruits, grains, seeds, nuts, and legumes may contain hundreds of different phyto-chemicals. The consumption of such foods containing these bioactive components can provide desirable health benefits beyond their natural properties when consumed in a consistent manner through diet. Alternatively, dietary supplements can be also supplied to consumers in a concentrated form to deliver a specific bioactive or a group of phytochemicals [4–7].

Further, the most critical step for the successful application of these metabolites in the market is to profile them with accuracy in different sources such as plant-based sources (fruits, vegetables, leaves, seeds, etc.). This requires using various sophisticated methods and techniques in order to boost their detection and use at a quicker rate for the development of novel medicines, nutraceuticals, chemical discovery, food safety, and quality.

Various analytical procedures have already been developed for the detection of these metabolites but due to a number of unparalleled advantages such as fastness, accuracy, intactness, magnetic resonance techniques, i.e., nuclear magnetic resonance (NMR), magnetic resonance imaging (MRI), and electron paramagnetic resonance (EPR) have fulfilled a significant role in determining structures and dynamics of various physical, chemical and biological systems in the field of food analysis (Figure 1). Hence, the purpose of this review paper is to highlight the advancing potential of these magnetic resonance techniques in the area of profiling of various plant-based natural constituents in order to explore their possibilities of utilisation in new research and development areas [8–10].

2. Nuclear Magnetic Resonance (NMR) as Potential Tool for Profiling

Nuclear magnetic resonance (NMR) is an efficient analytical technique used for solid or liquid materials that have grown in popularity in the field of food technology for the analysis and detection of a wide range of metabolites in fruits, vegetables, beverages, oils, meat, and dairy products. Although, the use of NMR in food was restricted earlier due to low-resolution moisture measurement but now NMR has expanded to encompass high-resolution investigations of

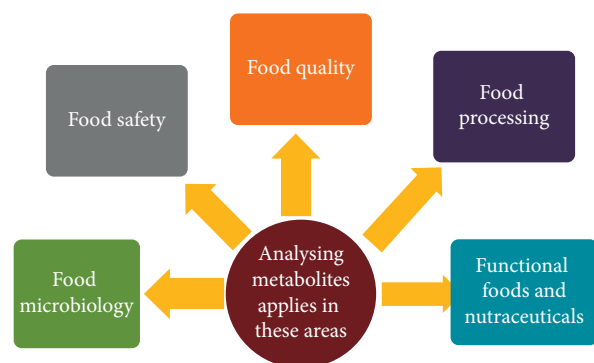


FIGURE 1: Various applications representing importance of analysing metabolites in areas of food technology.

liquid and solid-state matrices for a variety of applications such as quality assurance, sensory evaluation, authentication, classification, compositional assessment, and structural characterisation [11, 12]. A few other applications may also include, studying the food component interactions, molecular pathways, and the investigation of their nutritional aspects in relation to health [13, 14]. The growth of NMR-related food applications has been aided by the development of advanced multinuclear/multidimensional and solvent quenching NMR methods, as well as advancements in NMR hardware such as cryoprobes, high-throughput technologies, and user-friendly software. Other important considerations include the numerous advantages of NMR spectroscopy over other analytical techniques, such as accuracy and multiple sample detection, which make it a useful tool in the food industry and regulatory authorities to meet customer demands for safe and healthy meals. Furthermore, because of its nondestructive nature, high precision and repeatability have shown great promise in analysing multicomponent systems such as foods without the need for any separation or purification methods [15, 16].

2.1. Principle. The NMR phenomenon occurs when a spin nucleus is placed in a magnetic field and an energy transfer occurs between distinct energy levels corresponding to radiofrequency radiation. The NMR signal generated can be detected and processed in a variety of methods including Fourier transform, phase correction, chemical shift calibration, integration, and time domain experiment to provide a specific NMR spectrum for the nucleus of interest. Nuclei of various isotopes (e.g., ^1H , ^{13}C , ^6Li , ^{10}B , ^{11}B) have been studied out of which commonly used nuclei are ^1H and ^{13}C by high-field NMR spectroscopy. Proton is the initial and the most frequent atom to be used in NMR spectroscopy. It is also called hydrogen-NMR (^1H -NMR) that provides information about the different varieties of hydrogen present in the molecule and also gives information about its adjacent surroundings. Solid-state NMRs are used for chemical analysis to recognise any changes in the structure during phase transitions and different transformations in a solid state. The main technique frequently used in a solid-state NMR is magic angle spinning (MAS) which makes the resolution of the sample. Phosphorus is one of the isotopes

used to study the molecules and structures of different samples. Compound classes of phosphorus were identified which include orthophosphate diesters, polyphosphate, phosphonates, and orthophosphate monoesters. ^{13}C is an isotope of carbon that has a spin quantum number of 1/2 and is only 1.1% naturally present, and this isotope can be detected by ^{13}C -NMR and gives detailed information regarding the chemical structure of the organic compounds. ^{15}N NMR spectroscopy is widely used in the structural elucidation of proteins and genetic material (RNA & DNA). While the utility of ^{31}P in metabolomic studies is limited because most metabolites do not contain phosphorus atoms thus is used in studying a small number of important phosphorous-containing compounds such as phospholipids and nucleoside metabolites (ATP, GTP, NADP, etc.) involved in energy metabolism. A homogeneous magnetic field strength is required for most NMR investigations but the magnetic field is set to fluctuate linearly for MRI and diffusion coefficient (D) determination. Chemical shift, peak integral, coupling constant, line widths, length of relaxation, and the nuclear over-hauser effect (NOE) are all significant NMR variables to consider during detecting NMR phenomena [17]. NMR is classified into three groups based on the magnitude of the magnetic field, i.e., high field NMR (magnetic field strength $> 1.0\text{ T}$), midfield NMR ($0.5\text{ T} < \text{magnetic field strength} < 1.0\text{ T}$), and low field NMR (magnetic field strength $< 0.5\text{ T}$) [17, 18].

2.2. Types of NMR. NMR spectra can be one-dimensional (1D) with only one frequency dimension or multidimensional (2D) with several frequency dimensions. 1D and 2D experiments are extensively employed in food science applications. 1D experiment is being used to detect heteronuclei containing ^{13}C and ^{31}P compounds. Several components such as α -glucose, β -glucose, fructose, malic acid, and tartaric acid have distinctive NMR signals which can be easily traced. While the primary goal of 2D NMR investigations is to obtain information about nuclei in close proximity. Cross peaks (correlation peaks) with two frequencies indicate a correlation (coupling) between two distinct nuclei in a 2D experiment. 2D experiments can be homonuclear or heteronuclear including the connection of similar or distinct types of nuclei and are based on polarisation transfer from one nuclei to another [16, 19]. As compared to 1D NMR, they provide improved resolution due to two frequency dimensions. This is necessary, especially in complex molecules or with complex mixtures as in foods and bio-fluids. The identification and quantification of metabolites by NMR can be further classified into targeted and untargeted metabolomics which helps in providing accuracy from initial to final detection steps using analytical procedures to specific compounds and nonspecific analysis, respectively.

2.3. Metabolomic Investigation

2.3.1. Targeted Metabolomics. Targeted and semitargeted metabolomic investigations aim to identify and quantify a

specific collection of metabolites in biological samples with precision. Typically, these sets of metabolites are predetermined by the scientific question at hand or the size of the metabolite library that is available in the software used for data analysis. The resulting data sets are fed into statistical data analysis tools to see how well targeted metabolites contribute to the group separation between control and phenotype of interest. Correlations are further evaluated to understand the underlying metabolism differences between the groups. Metabolomics profiling by NMR has been often performed by 1D ^1H NMR experiments [20–22]. The advantages of 1D ^1H NMR are their quick acquisition time (usually less than an hour per sample) and excellent sensitivity (specific compound detection). Targeted metabolite identification is usually accomplished by comparing a metabolite mixture's 1D ^1H NMR spectrum to a 1D ^1H NMR spectral database and then validating these identifications on a selection of samples using 2D NMR methods [23, 24].

2.3.2. Untargeted Metabolomics. Untargeted investigations majorly aim on measuring and comparing as many signals as possible across a sample collection followed by using metabolomics databases to assign these metabolite IDs to their signals. Despite substantial progress in populating metabolomics databases with new compounds, a significant fraction of signals obtained in untargeted experiments remains unidentifiable with this method due to the lack of spectra in the databases. Untargeted studies particularly help in identifying unknown metabolites, especially including the study's biomarkers and unknown metabolites. Their identification has traditionally been accomplished by isolating the molecule in adequate quantities from extracts for comprehensive study using MS, NMR, X-ray, circular dichroism, and other analytical techniques [25, 26]. While this method has been demonstrated to be effective and total fractionation takes time. Furthermore, a low purification yield may prevent downstream structural elucidation for low-abundance metabolites. Structure elucidation can also be done in a mixed environment such as in a crude extract or a partially fractionated material. For unknown identification in mixtures, three approaches have been proposed. Tang and Hatzakis [27] tested the ability of 1D and 2D NMR for untargeted metabolite analysis of pomegranate juice cultivar discrimination and detection of adulterants. For this, NMR along with chemometrics and untargeted heteronuclear single quantum coherence (HSQC) were applied with NOHA (nested NMR by ordered acquisition using ^1H detection) pulse sequences. Several metabolites including sugars, organic acids, and amino acids were identified. Moreover, NMR could also successfully detect the adulteration of pomegranate juice with apple juice. The second technique, on the other hand, is based entirely on mass spectrometry and other techniques for detection. The third technique deals with NMR with experimental chemical shifts of unknown metabolites being successively allocated and deconvoluted using multidimensional NMR. The accuracy of these designations is confirmed by comparing them to quantum NMR chemical shift predictions [28, 29]. While

these MS and NMR-based techniques make structural characterisation easier but they are limited in their power due to the preference of using a single technique at a time over combination techniques.

2.4. Analytical Procedure. The samples used for NMR analysis must be representative of the total population and carefully chosen in order to minimise biasness and preserve a normal distribution. Accurate and precise documenting of sample identity and characteristics are critical in this or any other metabolomics study. In most cases, sample preparation includes an extraction phase in which compounds of interest are extracted from the food product using a suitable solvent (the choice of solvent depends on the compounds of interest) and/or a separation process, while additional steps such as drying and homogenisation may also be added if required [8]. To reduce biasness, sample randomization is also important during the preparation process. The sample is normally re-suspended in an appropriate NMR solvent that also contains a reference substance such as tetramethylsilane (for nonpolar solvents) or trimethylsilylpropanoic (for polar solvents) for solution-state NMR analysis. A set of free induction decay (FIDs) is obtained after the NMR acquisition. The spectrum processing for the conversion of FIDs to spectra [30] and identifying signals includes the standard techniques used in any other type of NMR experiment such as Fourier transform, phase correction, and baseline correction. Furthermore, all samples must have the same acquisition and processing of NMR spectra. After spectrum processing of raw NMR data, a preprocessing step is frequently necessary before performing multivariate statistical analysis in order to make the data from various samples analogous and assure appropriate analysis. The most common preprocessing procedures used on frequency domain data include spectrum alignment, sample scaling (normalisation), data scaling, and spectral editing. All of these procedures must be completed in the same manner as the sample preparation to avoid the introduction of variation [31].

3. NMR Approaches for Profiling of Fresh Fruits, Vegetables, and Grains

Fruits, vegetables, and grains supply essential nutrients needed for life as well as additional bioactive compounds for health improvement and disease prevention as these are good sources of a wide range of micronutrients and nonnutrient bioactive compounds including vitamins, phenolic compounds, colour pigments, minerals, and dietary fibres. More than 5000 unique phyto-chemicals are expected to have been identified in fruits, vegetables, and grains, although a major fraction of them is still unknown [32]. Metabolomics can be effective in finding novel bioactive phyto-chemicals from plant sources especially when combined with bioactivity studies. Although several analytical techniques can be used for metabolomics analysis, NMR spectroscopy has unquestionable advantages, particularly in terms of sample preparation and nondestructive

analysis. These properties make NMR a high-throughput, repeatable, and reasonably affordable technology [33, 34]. Hu et al. [35] conducted a study to detect adulteration in paprika powder with Sudan I, a carcinogenic industrial dye generally used for colouring clothes and wax. Both solid-state nuclear magnetic resonance (SSNMR) and NMR techniques were employed and compared for the determination of this adulterant in paprika powder which facilitated the rapid and accurate determination of Sudan I adulteration with the accuracy of 105 and 98 per cent, respectively. Recently, Chamley et al. [36] utilised proton NMR for the characterisation of individual components (mesocarp, exocarp, columella with placenta & locular tissue) of developing tomato fruits resulting in the quantification of 39 metabolites (14 amino acids, 6 soluble sugars, 11 isoprenoids, 4 organic acids and starch) in all tissues at various stages of fruit development. Similarly, metabolomic profiling of historical and modern wheat cultivars was conducted by Poudel et al. [37], using proton NMR spectroscopy. NMR profiling of wheat landraces indicated an increase in the number of amino acids and phenolics in modern cultivars along with the presence of 16 important metabolites belonging to four different chemical classes, namely, carboxylic acid (fumaric acid), amino acids (betaine, alanine, leucine, asparagine, isoleucine, threonine, valine, and tryptophan), phenolic acids (ferulic acid, chlorogenic acid, syringic acid & vanillic acid) and carbohydrates (maltose, sucrose, and glucose). Furthermore, Sket et al. [38] applied a constrained total-line-shape (CTLS) approach and HPLC-MS to ^1H NMR spectra for quantification and identification metabolites present in *Allium* species in order to determine functional ingredients of importance. Each of the sample contained a total of 18 metabolites including important sugars such as nystose, fructose, ketose, glucose, and amino acids such as phenylalanine, tyrosine, leucine, glutamine, glutamate, lysine, alanine, threonine, valine, ahydroxybutyrate, pyruvate and malic acid, flavonoids, glycosides mainly including kaempferol or quercetin derivatives. Recently, a study was conducted by Beteinakis et al. [39] on metabolic profiling of edible olives, specifically taking into consideration their geographical origin and methods of processing. NMR metabolic profiling was combined with multivariate analysis and obtained data revealed the presence of metabolites such as short chain fatty acids and triterpenic acids (Oleanolic acid and Mesalinic acid) which are linked to neuroprotective, antioxidant, antitumor, anticancer, and antidiabetic activities of table olives. Also, Xing et al. [40] utilised high field nuclear magnetic resonance (HF-NMR) spectroscopy for nutritional and quality evaluation of camellia and olive oil and low field NMR (LF-NMR) imagery for assessment of camellia oil when adulteration was done with corn oil. The presence of linolenic acid, linoleic acid, oleic acid, saturated fatty acids, and squalene was comparatively higher in camellia oil than in olive oil. In addition to this, LF-NMR imagery was found highly beneficial in identifying the adulteration ratio with 100 per cent accuracy indicating towards its potential application in determination of food authenticity.

4. Nuclear Magnetic Resonance for Profiling of Processed Food Products

NMR has been utilised in the food, beverages, cosmetics, and nutraceutical industries to monitor and control quality. Natural product profiling can be done in the same way. The whole process from sample collection to extraction, NMR measurement, data processing, and statistical analysis should be standardised to ensure reproducibility and dependability while minimising experimental errors. The NMR solvent influences the chemical shift locations of protons in phenolic compounds and other solvent effects, thus it is important to choose it carefully for ^1H NMR than for ^{13}C -NMR. When it comes to using NMR for profiling or metabolomics research, there are two main techniques. The first one is to collect and utilise only spectral patterns (chemical shifts and intensities) to compare and organise the samples. The compounds to be detected are not originally recognised in this method. This is frequently referred to as a chemometric method since statistical tools like principal components analysis (PCA) are employed. The second technique uses a targeted metabolomics approach in which a reference spectrum library is used by which certain chemicals known to be present in the extract are identified and quantified. These methods are not exclusive but with better statistical techniques and larger NMR spectrum databases, their usage can be increased in the future times for better profiling of products.

Further, to minimise the number of variables, statistical approaches known as chemometrics are used to reduce the quantity of data produced. Chemometrics is a collection of statistical techniques used to analyse huge amounts of chemical data such as NMR chemical shift peaks with the goal of obtaining insight into the samples properties through graphical depiction. It is an appropriate technique for NMR which generates a lot of data because it can handle enormous volumes of data (chemical shifts). This may be used to discover grouping patterns, quality control, and standardisation of samples of natural products. Since the year 2000, when chemometrics was first used for NMR, progress has been quite rapid. Chemometrics has been used to categorise entire plant samples according to species, provenance, processing treatment, age, and different quality characteristics based on their NMR patterns. NMR spectroscopy can also be used in honey analysis to determine its botanical and geographical origin. PCA (principal component analysis) and general discriminant analysis (GDA) were not able to group samples according to their botanical origin by using ^1H NMR data. The ^1H -NMR-based metabolomics approach also allowed for simultaneous qualitative analysis of green tea leaves in which amino-organic acids and phenolic metabolites were detected in the spectra. Green tea (*Camellia sinensis* L.) leaves located in different areas of the world contained characteristic amino acids, i.e., theanine and phenolic compound, i.e., epicatechine derivatives [41, 42].

A study on quality evaluation of different varieties of dry red wine based on nuclear magnetic resonance metabolomics revealed a significant difference in metabolites in

different wine samples of different years (2009–2012). The quality evaluation of different varieties of dry red wine (Merlot, Cabernet Sauvignon, and Cabernet Gernischt) was conducted by Hu et al. [43] using 1D NMR metabolomics and multivariate statistical analysis methods. Their results indicated the presence of 8 different metabolites including glycerol, malic acid, proline, alanine, ethyl acetate, lactic acid, succinic acid, and gallic acid with glycerol being the most abundant metabolite present in the highest concentration in Merlot wine. The highest gallic acid content was found in Cabernet Sauvignon wine whereas, Cabernet Gernischt had the highest concentration of lactic acid, alanine, proline, and malic acid (Figures 2–4). Thus, this finding by ^1H NMR may offer advice for the consumers to choose the suitable wine for their needs. Various other latest applications related to NMR for the profiling of different products are given in Table 1.

5. Magnetic Resonance Imaging for Structural Elucidation of Food Products

In the past centuries, countless ways have been devised to measure the quality-related attributes of the food which help in maintaining the overall quality and shelf life of food products. But now-a-days, advancements have been there on developing sensors for real-time with nondestructive sorting [61]. Quality inspections are still done manually in the food industry by experienced inspectors which are time-consuming, costly, laborious, and intrinsically unreliable owing to its subjective character. The introduction of computer-based imaging techniques has been necessitated by an increase in demand for objectivity, consistency, efficiency, and recently, computer vision-based image processing techniques have been rapidly developed which can quantitatively characterise complex size, shape, colour, and texture properties of foods [62]. Technologies that produce pictures can provide a new perspective from which the food processor can investigate into the structure and provide guidance throughout the quality assessment and inspection process. NMR and the images formed by it known as magnetic resonance imaging (MRI) are the most powerful, nondestructive analytical techniques that enable studies of the molecular structure through measurement of the interaction of an oscillating radiofrequency electromagnetic field with a sample immersed in a strong magnetic field (MRI). However, the development of alternative MRI applications based on nuclear magnetic resonance principles is underway, that is, nondestructive and may be used to assess essential quality characteristics of food items without intrusive testing. So, as this new noninvasive technology advanced slowly but steadily, it not only strengthened medical imaging but also invaded other fields of study such as agricultural sciences particularly in the food industry. The beginnings of NMR date back to the mid-20th century with Bloch and Purcell and quickly used extensively by chemists and biologists. However, until the end of the 1980s, NMR was not widely used in food science and MRI basically developed for medical field analysis [19, 63–67].

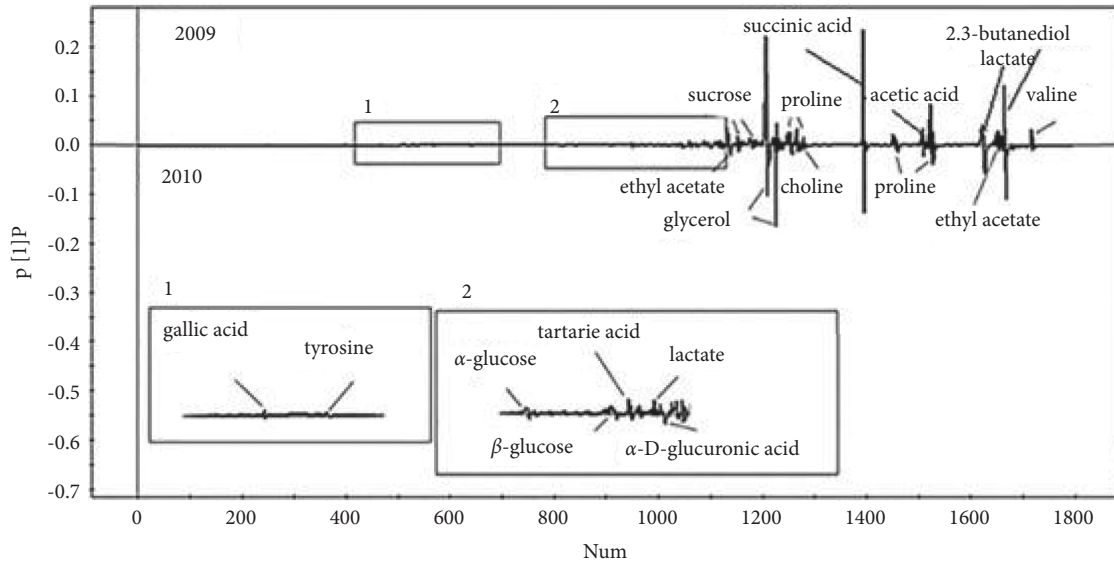


FIGURE 2: ^1H NMR spectra of 2009 and 2010 vintage cabernet sauvignon wines. Source: Hu et al. [43].

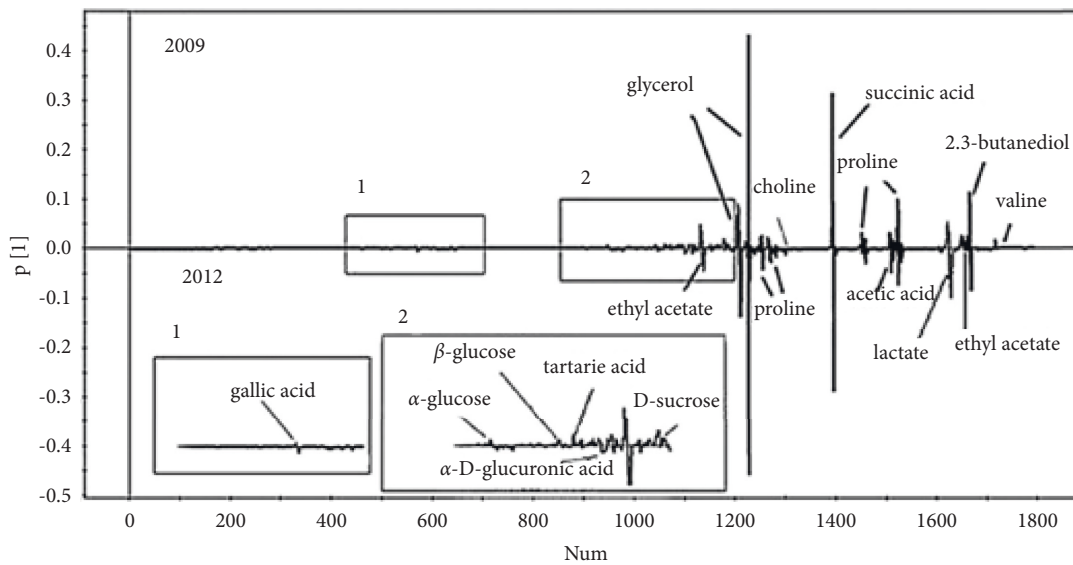


FIGURE 3: ^1H NMR spectra of 2009 and 2012 vintage cabernet sauvignon wines. Source: Hu et al. [43].

MRI is a nondestructive and noninvasive technique that can be used to acquire two-dimensional and even three-dimensional images of biological products even if they are in opaque systems. The signal of each component depends on the physical properties of the sample such as proton density, relaxation times, temperature, diffusion, flow, and local differences in magnetic susceptibility. The information within the images can then be manipulated and used for spatially resolved measurements of concentration, structure, temperature, velocity, and diffusivity. In contrast to the medical applications of MRI which are mainly based on a qualitative approach, this method may be used to investigate the internal structure and physical condition of water in fruit and vegetable tissues in a nondestructive and noninvasive manner [68]. Variations in tissue characteristics cause changes in the

MRI pictures because the NMR parameters are sensitive to plant tissue shape and composition. At both the cell and macroscopic levels, MRI may thus give both indirect and direct information. MRI has been used to gather data on fruit macrostructure and physiological changes in fruit tissues throughout development, ripening, and storage as well as to assess internal quality parameters like browning [69].

This technique gives results in the form of images, typically used to get high-resolution two or three-dimensional pictures determining the interior condition of an item from the interior of food materials. Various imaging techniques show various aspects of the biological organisms depending on the features of the imaging equipment, the competence of the operator, and compromises with parameters such as radiation exposure and imaging duration,

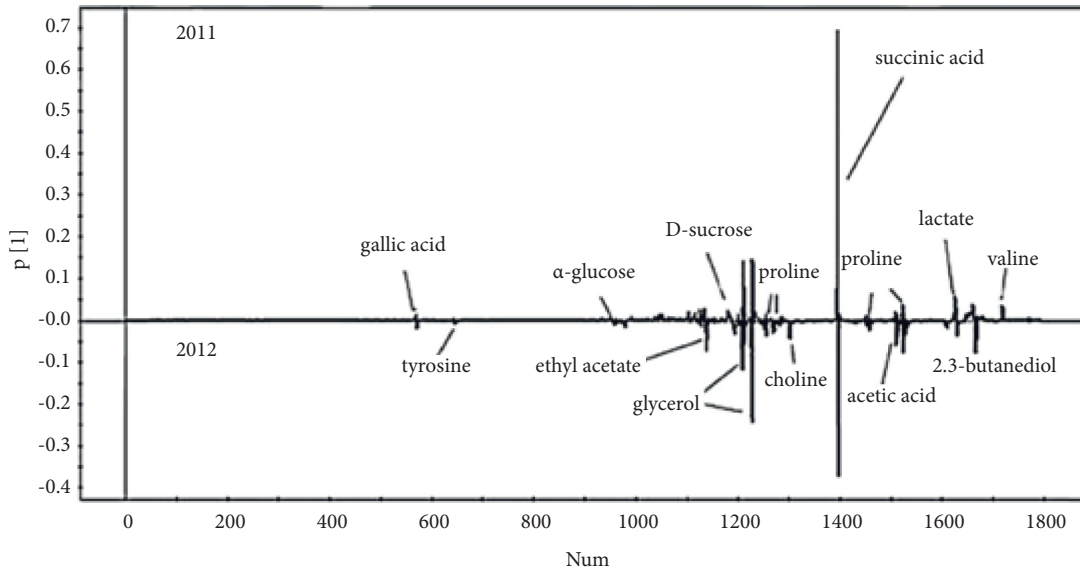


FIGURE 4: NMR Spectra of 2011 and 2012 vintage cabernet sauvignon wines. Source: Hu et al. [43].

the range in picture quality and structure visibility can be substantial for each approach [70].

5.1. Principle of MRI. Due to the intrinsic mechanical characteristics of the specific image of atomic nuclei in a magnetic field, MRI has become far more popular in the food service industry as it evaluates food quality by effectively depicting their structural information. A proton in the hydrogen nucleus for example behaves as a spinning charged particle with rotational momentum, producing a polar field [71]. Pure liquids, solutions, gels, emulsions, foams, suspensions, and solids, all of which include different quantities of water are examples of food that can be imaged by this technique. From a chemical perspective, food is made up of water, carbohydrates, lipids, and proteins. As a result of the physical and chemical diversity in food, many NMR methods may be used in research such as solution NMR imaging as well as solution and solid-state spectroscopy [72]. The amplitude of a nucleus' magnetic moment, the natural abundance of nuclei, and the strength of the applied magnetic field among other things determine its sensitivity to NMR spectroscopy. Food molecules contain NMR-active nuclides such as ^1H , ^{13}C , ^{31}P , and ^{23}Na . When hydrogen ^1H is abundant, it has the highest receptivity, whereas carbon ^{13}C has the lowest receptivity of 1.7104 relative to hydrogen. The ^1H nucleus which consists of a single proton is an ideal candidate for using MR techniques to explore the body for a variety of reasons. It has a spin of 1/2 and is the most common hydrogen isotope. It has one of the greatest sensitivities to a magnetic field found in nature. Also, as the body is made up mostly of water and fat, both of which include hydrogen and normal tissues may naturally produce a considerable MR signal [73].

At various magnetic field strengths, an investigation was conducted on the effect of magnetic susceptibility in homogeneities and proton density measurements in white

button mushrooms. It was demonstrated that MRI mapping with low fields and short echo durations offers numerous benefits for quantitative imaging of water balance and mapping of tissues with high sensitivity deformations. Furthermore, for the measurement of apparent microporosity spatial distribution, susceptibility effects have recently been proposed. The technique is based on the magnetic susceptibility differences between gas-filled intercellular gaps and their aqueous environments within fruit tissues. The approach was evaluated at two magnetic fields and compared to X-ray microtomography studies of local porosity, demonstrating that MRI is efficient in detecting the distribution of apparent microporosity in fruit [74].

6. MRI for the Profiling of Different Food Components

MRI offers the benefit of allowing a wide range of measures to be taken which not only aid in the evaluation of maturity and quality characteristics in fruits, vegetables, and other food items but also aid in the study of the physiological processes that promote them. As a result, the capacity of MRI to operate in a fully nondestructive manner and to record molecular dynamics using multiple contrast mechanisms has sparked the creation of several applications in a variety of disciplines. Windt et al. [66] were able to demonstrate that most of the water translocated into the tomato fruit travels through the xylem and not the phloem, thereby resolving a long-standing difficulty in modelling fruit growth. This relatively new imaging technology has found value in food engineering because it gives a rapid, direct, and most importantly, noninvasive, nondestructive means of detecting not only the amount of water present but also the structural dynamic characteristic of water [75, 76]. Mainly because water is the main building ingredient of many food materials, the MRI technique has a wide range of applications.

The study of specific aspects of fruit quality such as at different quality stages has also found uses in magnetic

TABLE 1: Latest applications of NMR for the profiling of various fresh and processed products.

Plant-based raw and processed products	Type of NMR/approach	Key findings	Reference
Honey	NMR + chemometrics	Detected saccharides, amino acids, aldehydes, aliphatic and aromatic compounds	[41]
<i>Moringa oleifera</i> leaves	^1H NMR	Successfully analysed metabolites at different concentrations and highlighted the antioxidant potential for high immune system	[44]
Purple carrot	NMR- ^1H TOCSY with PCA	Detected various bioactive compounds amino acids, aromatic amino acids, phenylpropanoid, GABA, chlorogenic acid, omega-3 fatty acids, etc. Identified several biomarkers to find optimal harvest time of purple carrots	[45]
Beer	^1H NMR + chemometrics	A clear discrimination of compounds from 31 samples of beer Both targeted and untargeted approaches gave high resolution of metabolites	[46]
Legume sprouts	^1H NMR + chemometrics	32 metabolites were identified to different classes, i.e., fatty acids, sugars, amino acids, organic acids, sterols, alkaloids, and isoflavonoids	[47]
Pineapple waste extracts	^1H NMR	Successfully investigated metabolite profile of pineapple peel, crown and core extracted from different ethanolic ratios Also identified toxic compounds and co-relation between plant metabolomics and their bioactivity	[48]
Short cake biscuits	NMR-low field	Indicated a reduction in dynamics of water molecules bound to polymer matrix Rheological characteristics such as reduction in hardness of cookies was analysed by NMR imaging	[49]
<i>Arabidopsis thaliana</i>	NMR-MS	Identified unknown metabolite accurately such as glucosinolates and glucoraphanin	[50]
Guava leaf extract	^1H NMR + principle component analyses	High degree of variation detected by PCA analyses of ^1H NMR spectra Signals detected aliphatic and aromatic regions suggesting both primary and secondary metabolites such as quercetin, ferulic acid, and gallic acid	[51]
<i>Coffea arabica</i> roasted beans	^1H NMR and static headspace gas chromatography (SHGH)-MS	Detected 20 water-soluble compounds and 43 volatile compounds such as n-methyl pyridinium, formic acid, caffeine, choline, propionic acid, 2-furyl methanol	[52]
<i>Corylus avellana</i> leaves (hazelnut)	^1H NMR and principal component analysis, partial least square-discriminant analysis (PLSDA)	30 metabolites were detected such as valine, kaempferol, giffonin, quercetin, and carpinotriol	[53]
<i>Anogeissus leiocarpus</i> DC stem bark	^1H NMR-HPLC-DAD-MS	Detected 59 compounds including 43 ellagitannins and 16 triterpenoids such as roburin, vescalin, castalin, etc.	[54]
Apple	^1H NMR	Identified 4 sugars (fructose, glucose, sucrose, xylose) and 6 amino acids (Alaline, asparagine, asparatate, γ -amino butyric acid, isoleucine and valine) and 9 organic acids (acetic, citric, etc.)	[55]
Commercial date palm	^1H NMR + UPLC-MS	35 metabolites were detected in the commercial product such as quercetin, procyanidins, kaempferol, catechin, salicylic acid, disaccharides, procatechuic acid, and glyceric acid.	[56]
Seabuckthorn	^1H NMR-PLSDA	Numerous new bioactives such betaine, spermidin, adonitol, beta-alanine, creatine, xylitol, taurine, etc. other than 100 bioactives were found	[57]
Sugarcane juice	^1H NMR	42 compounds such luteolin, apigenin, gentsic acid, malic acid, formic acid, aspartate, fatty acids, valine, etc. were successfully detected.	[58]

TABLE 1: Continued.

Plant-based raw and processed products	Type of NMR/approach	Key findings	Reference
Mango	^1H NMR + PCA	Detected compounds such as malonic acid, phenyl alanine, glutamine, lysine, ethylisobutanote, and phosphocholine	[59]
Rice (white, black, and red)	^1H NMR and PLSDA	Tyrosine, gallic acid, ferrulic acid, sinapic acid, xylose, galactitol, tocopherol, oryzanol, linolenic acid, oxoglutaric acid, α -aminobutyric acid, etc. were detected	[60]

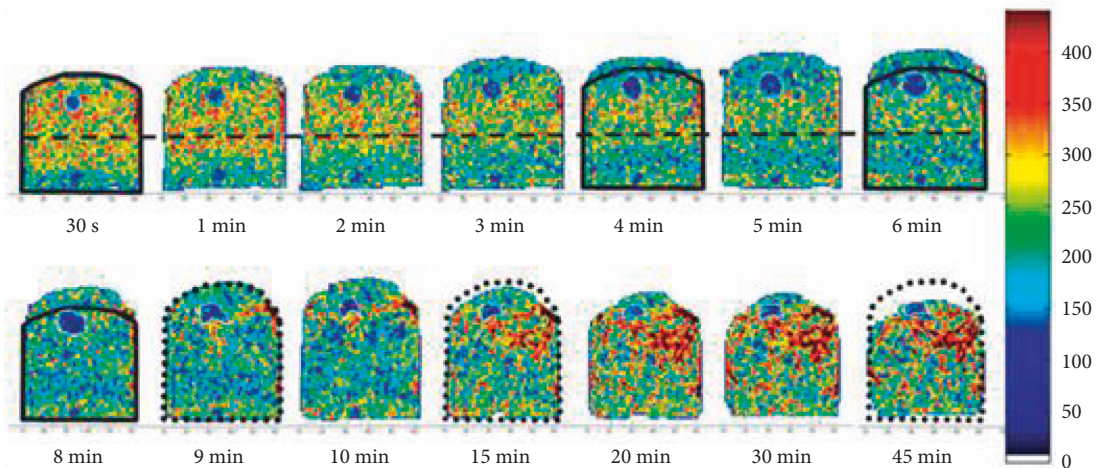


FIGURE 5: Magnetic resonance images (MRI) of bread acquired during baking. The superposition of the boundaries of the image taken at 9 minutes on photos taken at 15 and 45 minutes of baking emphasised the overall bread bulk (dotted black line). Source: Wagner et al. [70].

resonance imaging. The use of MRI sequences with an optimised acquisition time, often on a second-to-minute scale is also required for dynamic imaging of cereal products in the processing stage, however, that the water transport in rice kernels observed during cooking may differ depending on the sequence duration. The poor signal-to-noise ratio which is explained by the low relaxation periods and finally the presence of gas is another limitation in the design of the sequence parameters (expanded cereals). Comparing MRI data to models of heat and transport combined with deformation and chemical reactions typically requires quantification, a necessary step on the way to a better understanding of the phenomenon. However, numerous variations in temperature, water content, ice melting or freezing, protein denaturation or gelation, starch gelatinisation or retrogradation, and bubble expansion or collapse frequently impair MRI measurement. Any referential relationship between the MRI signal and one of these variables should be established ideally by comparison with traditional measuring techniques (e.g., water content assessed globally or locally). A combination of NMR and MRI data might potentially aid in identifying the most sensitive MRI signal components [77, 78]. An oven dedicated to continuous MRI measurement was constructed and tested during a study in which conventional response parameters measured on bread during baking and after cooling (temperature, apparent density) proved to be consistent with findings reported in the literature for similar products. This confirmed that baking

conditions produced with this MRI oven were comparable at least with traditional, convection ovens and that conclusions drawn from future MRI studies could be generalised to some extent. This study also highlighted how sensitive the MRI signal is for many factors which vary during baking. This feature can be considered as part of a pool of information but it is also difficult to directly interpret the variations in the MRI signal observed during baking as shown in Figure 5 [79].

7. Electron Paramagnetic Resonance for Food Products

Electron paramagnetic resonance (EPR) spectroscopy is a method for detecting unpaired electrons produced by radicals such as those produced by paramagnetic ions or by irradiation in an external magnetic field. In 1955, the first EPR spectrum from an irradiated biological material was published. Following this, in 1971, EPR was thought to be a method for detecting irradiated food, and later, the European Committee for Standardization approved EPR as a standard procedure. European Standards for crystalline sugar, cellulose, and bone are based on electron spin resonance. EPR spectroscopy is a sensitive and adaptable method for studying molecules with unpaired electrons such as organic radicals and paramagnetic metal ions. Food degradation is mostly caused by oxidation events as seen by the generation of organic radicals in foods. Also, food metal

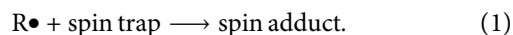
ions can stimulate the oxidation of food components by activating oxygen to form reactive oxygen species (ROS). In addition to this, EPR can also be used to assess the shelf life and stability of food. Acceleration of radical production and degradation in food is required to conduct such investigations [80]. For the creation of radicals in meals, several methods have been used, including Ultraviolet rays, microwaves, heating, γ -radiation, and the addition of oxidants. EPR can directly detect stable organic radicals such as semiquinone and tyrosyl radicals [81, 82]. Spin traps, on the other hand, are used to detect transient radicals which may then be quantified via EPR spectroscopy. The short-lived radicals' lives can also be extended by rapidly freezing the samples after they've been generated. In addition, short-lived radicals can be detected using time-resolved EPR. By monitoring the EPR signal vs. time, valuable information about the mechanisms involved in these events can be obtained [83].

7.1. Principle and Working of EPR. Electron Paramagnetic Resonance is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field. The applied magnetic field generates discrete orientation and this orientation difference is physically equivalent to a separation of energy levels. Resonance absorption of electromagnetic radiation (microwave energy) occurs when the applied microwave energy exactly matches the energy level separation. Radiation-induced paramagnetic centres (e.g., radicals) are detected using EPR. A strong external magnetic field causes a difference in the energy levels of electron spins, $m_s = +1/2$ and $m_s = -1/2$, resulting in microwave energy resonance absorption. Irradiated samples are identified by determining the g value of the EPR signal ($h\nu/\mu_B B_0$, where ν is the microwave frequency, h is Planck's constant, B_0 is the magnetic field, and μ_B is the Bohr magneton). The first derivative of the absorption with respect to the applied magnetic field is used to represent the EPR spectra [83]. If compared to the shelf life of the food, radiation-induced paramagnetic species can remain stable in the stiff and dried sections of a food sample for a long time. The equipment of EPR is shown in Figure 6.

7.2. EPR Functioning and Measurement. The components and functioning of an EPR spectrometer are discussed as follows:

- (1) Source: A gun diode is used to generate monochromatic MW radiation in the 9.75 GHz (known as the X-band) region. Other regions of interest are the L-band (w1.1 GHz), S-band (w3.0 GHz), Q-band (w34.0 GHz), and W-band (w94.0 GHz). In the Gunn tube, the source of MW radiation is a Gunn oscillator (a solid-state device). It also contains those components which control or measure the frequency and the intensity of the MW beam.
- (2) The magnet system: It is an electromagnet providing a stable, linear variable and homogeneous magnetic field of arbitrary magnitude.
- (3) The cavity system includes the components which hold the sample and also direct and control the MW beam to and from the sample.
- (4) The detector and modulation systems monitor, amplify, and record the signal.
The MW radiation travels down a waveguide (a type of RF pipe) to the sample, which is held in place in a MW cavity held between the poles of two magnets. The frequency of the MW remains constant, while the impedance of the cavity can be changed mechanically (screw) and electrically. Spectra are obtained by measuring the absorption of the MW radiation while scanning the magnetic field strength. The absorption of this photon by the sample will be indicated by a change in the detector current.
- (5) Phase-sensitive detector: As mentioned earlier, the direct detection of the absorption signal is possible only for samples containing a high concentration of unpaired electrons. Noise components occur over a wide range of frequencies that appear with the signal, making its detection difficult. This is overcome by field modulation using a phase-sensitive detector technique. It utilises small-amplitude magnetic field modulation that is used to limit the noise-contributing component to frequency. Modulation at the commonly used frequency of 100 kHz is achieved by placing small Helmholtz coils on each side of the cavity along the axis of the static field. This gives the signal as a first derivative.

EPR can be used as a direct or indirect approach for detecting and identifying free radical metabolites. On fungal spores of *Penicillium digitatum*, a biological semiquinone radical with a line width of around 5g and g -value of approximately 2.004 and can be kinetically analysed *in situ* at the time of atomic oxygen-produced plasma electric discharge in real time, and the degradation of the EPR signal is probably related to the deactivation of the fungal spores [85]. Nonthermally processed (PEF, HPP, etc.) raw horse meat when exposed to nitrogen, atomic hydrogen, and oxygen gives rise to characteristic EPR signals from peroxy radical (RO_2^\bullet) and Fe^{3+} state on myoglobin or haemoglobin [87], which can be employed as a marker of a balance between microbe inactivation and food nutritional status deterioration [88]. For relatively long-lived radical species, direct EPR is conceivable, while spin labelling and spin trapping techniques are used for indirect EPR. The creation of long-lived and EPR-detectable spin adducts as a result of the reaction of a short-lived reactive free radical R^\bullet with a diamagnetic molecule is the basis of the spin trapping technique [89–91].



The spin adduct (typically nitroxide) should be a radical product with a lengthy half-life. In an EPR spectrum, the signal intensity of the spin adducts is proportional to the concentration of the generated free radicals R^\bullet . PBN (N-tert-

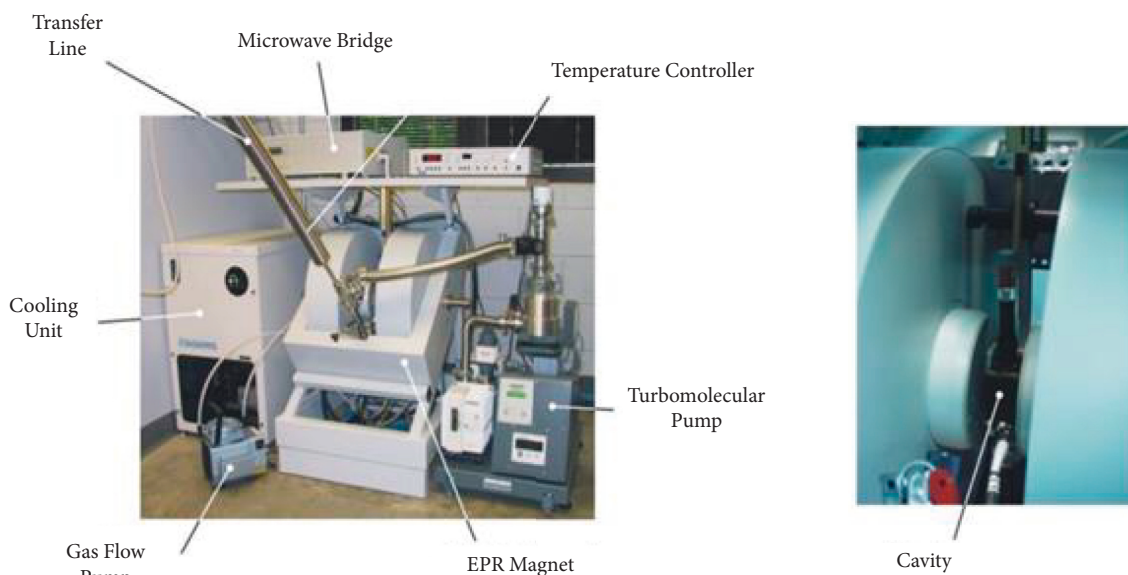


FIGURE 6: Electron paramagnetic resonance equipment (source adapted by: van der Kraaij et al. [85]).

butyl- α -phenylnitron) and DMPO (5,5-dimethyl-1-pyrroline N-oxide) are popular spin traps used in biological studies. For popular spin traps, information on the hyperfine splitting of the spin adduct is widely known [91]. The splitting patterns in spin adduct EPR spectra can reveal important details regarding the identity and structure of trapped radicals. The spin trapping approach was first designed to investigate biological molecules with extremely reactive and short-lived hydroxyl ($\text{HO}\bullet$) and superoxide ($\text{O}_2^{\bullet-}$) radicals as well as radical production on lipids, polysaccharides, and proteins. An EPR of different herbs [92] i.e., caraway, curry, curcuma, and cardamom revealed free radical concentrations of sterilised vs. nonsterilised samples. The type of radicals used and the spin adducts used to determine the trapping efficacy and stability of the resultant adducts.

7.3. Evaluation of the Physico-Chemical and Nutritional Properties of Food. The identity, behaviour, environment, and quantity of radical species can all be determined via EPR spectroscopy. ROS and RNS are important regulators of cellular physiological and pathological pathways. Different EPR approaches are available for detecting dissolved oxygen concentrations as well as free radical scavenging, stability, and chelating activity of dietary components. The ease of detection and identification of free radicals created by chemical or biological systems by examining the spectrum of a spin adducts is one of the key advantages of EPR mentioned in the literature. Moreover, it also enables the quantification of free radicals by comparing peak areas to those acquired from stable radicals as well as kinetic analyses and the determination of a free radical's generation and elimination velocities [93]. However, the technique has some significant flaws such as the inability to identify a free radical when it reacts instantly with a molecule other than the spin-trapping agent. Furthermore, when a reducing agent is

present, spin adducts can be neutralised and if a spin adduct is dissolved, a new spin adduct can be produced, increasing the difficulty of identifying free radicals. When the hyperfine coupling constant, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO), is the only EPR parameter determined for a spin adduct, it is also difficult to determine the electron distribution and molecular structure of the free radical. It is worth noting that EPR techniques are only qualitative not quantitative in some situations [94–96]. ESR has been used to investigate irradiated fruits, vegetables, tea leaves, seeds, spices and herbs, meals containing bones, crystalline sugar, sauces, and beverages [97, 98]. Free radicals in lyophilised plants and based products such as coffee, cocoa powder, and chocolates have also been studied using EPR. A depiction of EPR graphs of phenols during wine making process is shown in Figure 7 [83].

8. Comparison of NMR Techniques with MS/HPLC

NMR spectroscopy and mass spectrometry (MS) are the principal methods of metabolomics, the branch of “omics” that deals with small molecules. Despite the fact that MS methods such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are the most widely employed in metabolomics and NMR nevertheless has a number of significant benefits. NMR, in particular is nondestructive, unbiased, and quantitative, requiring no separation or derivatisation, and may be used to study substances that are difficult to test using GC-MS or LC-MS. GC-MS, e.g., frequently necessitates the derivatisation of substances like sugars and amines. Sample preparation, chromatographic separation, precise experimental and ionisation settings, apparatus, and operator competence are all required for LC-MS which makes it difficult to standardise MS analysis. NMR, on the other

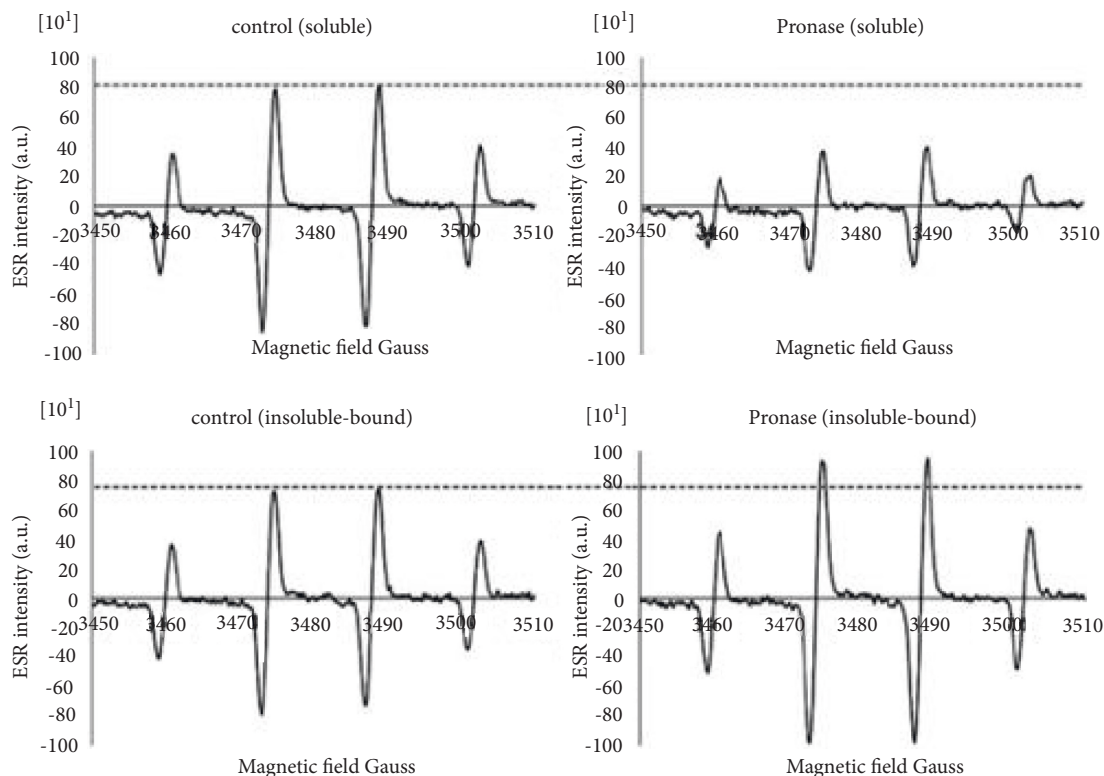


FIGURE 7: ESR signals of soluble and insoluble-bound phenols affected by pronase enzymatic pretreatment on winemaking by-products. Higher the ESR signal, lower the scavenging activity as demonstrated by the content of DMPO-OH adducts. Source: Ishikawa et al. [83].

hand, does not need extensive sample preparation and fractionation and is extremely repeatable which may offer both qualitative and quantitative information on a wide range of chemical substances [99]. The standardisation of the NMR procedure will increase the use of NMR a tool for profiling natural product extracts even further. NMR is not appropriate for the identification of trace components since it can only identify substances down to 0.1 per cent. The sensitivity of NMR is lower than that of MS which can identify molecules down to parts per million (ppm) levels [100–103]. NMR and MS are considered complimentary methods due to the different benefits of each approach. However, it should also be noted that NMR still offers some unique advantages and fills some important holes that cannot be filled with other metabolomics technology platforms. An investigation was carried out by Nkobole and Prinsloo [104] on two cultivars of *Amaranthus* spp. (i.e., wild and cultivated) by ^1H NMR and LC-MS-based metabolomics revealed that NMR analysis successfully detected the presence of various sugars, amino acids, and metabolites such as maltose and sucrose, trehalose, trigonelline, lactulose, betaine, valine, fumarate, formate, and kynurenine in both the varieties. It was found that the cultivated species had proline, while the wild species had leucine in dominant proportion. On the other hand, LC-MS analysis revealed the presence of rutin-2-phenyl ethenamine and amarantussaponin I in both wild and cultivated species, while chlorogenic acid was identified only in cultivated species. On the contrary, L-tryptophan, kaempferol, phenylalanine, and quercetin were detected only in the wild cultivar. The study

highlights the different aspects of ^1H NMR and LC-MS with their unique set of advantages and disadvantages. NMR allows a wide range of metabolites to be identified simultaneously, offering an accurate and easily reproducible representation of the metabolic profile of plants. However, compared to Liquid Chromatography-Mass Spectrometry (LC-MS) or HPLC, NMR is less sensitive in targeted metabolite identification. MS is preferred for its excellent sensitivity, high specificity, and simplicity without elaborate sample preparatory procedures in specific areas of analysis, while HPLC can be used to reduce the complexity of NMR spectra and to increase the signal strength of trace-level compounds. Thus, it can further enhance the resolution of unknown metabolites, particularly for isomer metabolites and poor chromatographic separation. Further, NMR is solely still preferred and rising in interest due to its high reproducibility, nondestructive approach, and minimal sample requirement and these techniques can be efficiently combined for better profiling of known as well as unknown metabolites efficiently.

9. Combination of NMR and Other Techniques (GC/LC-MS)

A hybrid method combines two or more analytical techniques to aid in the detection and quantification of components in a mixture. Various NMR combination approaches have been proposed to enhance the aspect of detection sensitivity, particularly with MS. Simply said, NMR identifies the most abundant metabolites, whereas MS

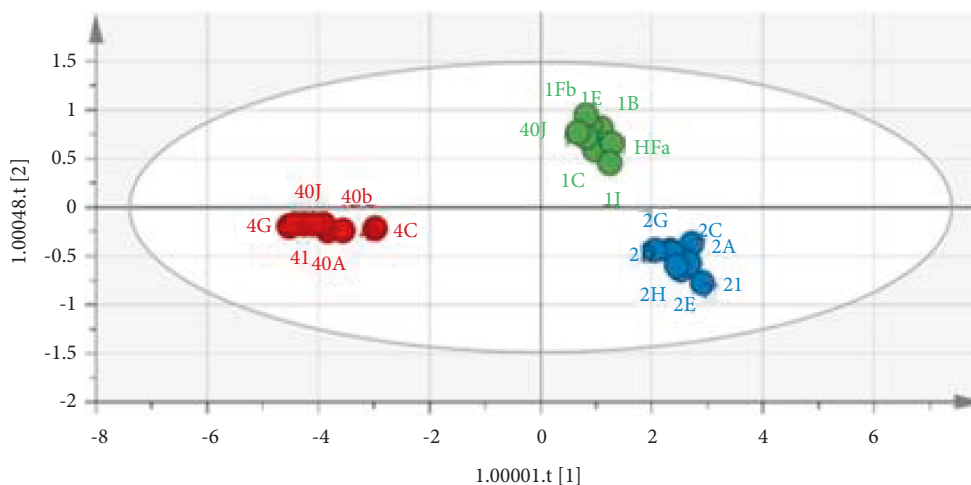


FIGURE 8: Score scatter plot of metabolites of combined OPLS-DA and LC-MS of *A. cruentus* leaf extracts (wild and cultivated). Blue = collected from the wild, green = cultivated at mothong in the shade net, and red = cultivated at mothong in the open field [104].

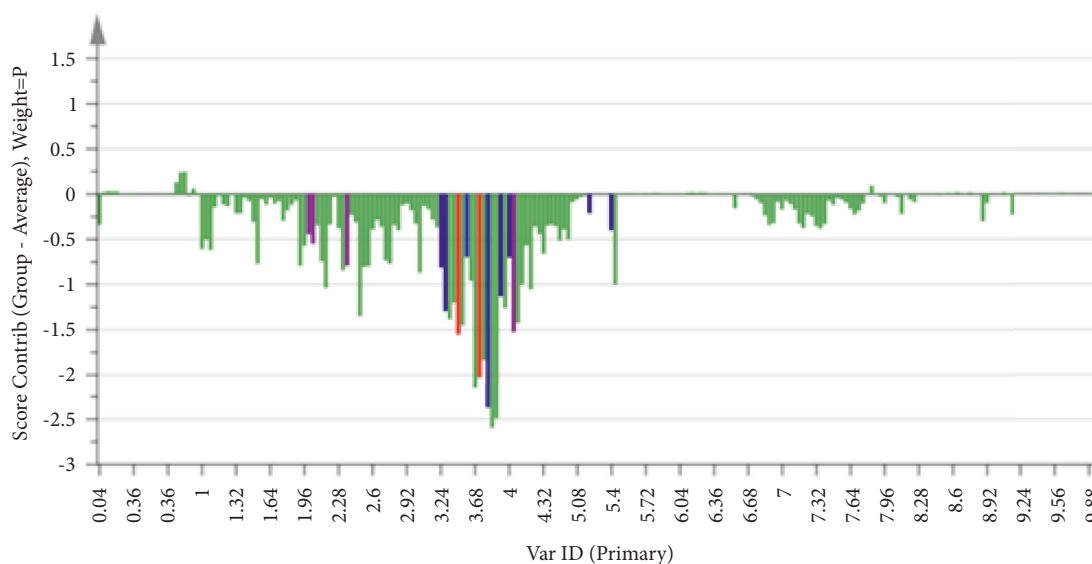


FIGURE 9: The blue, red and violet bars represent NMR regions that are associated with maltose, sucrose and proline, respectively. There may be overlaps between NMR regions of maltose, sucrose and maltose [104].

detects those which can be ionised readily. For example, NMR involves minimal sample treatment but MS metabolomics necessitates chromatography due to the high narrow molecular weight range of metabolites [102]. Derivatisation of metabolites is not consistent and column reactivity is also low. To name a few problems, chromatography systems have nonuniform metabolite derivatisation, poor column recovery, breakdown during derivatisation, ion-suppression due to the co-eluent matrix and possibly mismatched retention periods. Small molecules too have variable thermal stability which can lead to metabolite loss and an incorrect build-up of breakdown products at temperatures often used in GC [103]. In contrast, NMR lacks the sensitivity to detect metabolites in the sub-micromolar range (1 M) and has a limited spectral resolution resulting in peak overlap. MS offers a higher

resolution and a wider dynamic range than NMR and dynamic range. While poor sensitivity will remain a drawback of NMR spectroscopy in comparison to mass spectrometry, new advances in probe design, magnet design, magnet field strength, pulse sequences, and sensitivity enhancement approaches are bringing NMR closer to the sensitivity claimed by several MS systems. Some of the most common hybrid analytical methods are gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) [50], gas chromatography-infrared spectroscopy (GC-IR), and liquid chromatography-nuclear magnetic resonance spectroscopy (LC-NMR). Various studies have previously been discussed which significantly highlight the importance of using hybrid methods for a better future outcomes in the field of metabolomics as shown in Figures 8 and 9 [54, 104].

10. Limitations and Future Perspectives

The fundamental drawback of NMR spectroscopy has always been its low sensitivity. While NMR has no theoretical sensitivity limit since the number of acquisition scans may always be increased and the realistic (time-dependent) detection limits are still in the low-micromolar to high-nanomolar range. Despite substantial signal amplification with stronger magnetic fields, cryoprobes, and digital signal processing, many essential low-abundance metabolites remain undetectable with NMR technology today. The procedure of elucidating the structure of any tiny organic molecule is a tough task therefore it is not unexpected that spectroscopists arrive at different findings. Moreover, the high cost of NMR, MRI, and EPR equipments also limits its applications especially a small scale. For accurate results and improving its time efficiency, there shall be a requirement of high manual labour with expertise to prevail its further use with respect to its economic aspects [50, 104]. The combination of techniques has however helped in improving the issue of sensitivity issue but these challenges must be looked upon for future needs and high outputs in research and development as well as industrial applications.

11. Conclusion

Structural elucidation of secondary metabolites has gained importance in the recent times due to their high significance in our daily life. In this context, plants are one of the major abundant sources of metabolites consumed by almost 80% of the world's section. Magnetic resonance techniques such as NMR, MRI, and EPR are major detection techniques with high accuracy and precision and are able to analyse hundreds of metabolites without damaging the sample in one go. These techniques not only help in the detection of important metabolites but also help in discovering new compounds, antinutritional factors, free radicals detection, and for new drug development. It is, therefore, important to consider these emerging technologies for the future and work on their advancements to overcome their limitations to get benefit at the extremity for new advances and developments.

Data Availability

Available data were collected from the research publications collected from public domain to compile the review.

Disclosure

This is not a research study. However, the facilities of Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India, has been utilized for the collection of relevant literature.

Conflicts of Interest

The authors declare no conflicts of interest.

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