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STUDY OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY WITH APPLICATIONS: A COMPREHENSIVE REVIEW

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❖ ABSTRACT:

Nuclear magnetic resonance (NMR) spectroscopy is one of the most significant analytical techniques that has been developed in the past few decades. A broad range of biological and non biological applications ranging from an individual cell to organs and tissues has been investigated through NMR. Various aspects of this technique are still under research, and many functions of the NMR are still pending a better understanding and acknowledgment. Therefore, this review is aimed at providing a general overview of the main principles, types of this technique, and the advantages and disadvantages of NMR spectroscopy. In addition, an insight into the current uses of NMR in the field of medicine and dentistry and ongoing developments of NMR spectroscopy for future applications has been discussed.

❖ Keywords:

Nuclear Magnetic Resonance, metabolism, multiple sclerosis, spectroscopy, Magnetic Resonance Imaging,

❖ INTRODUCTION:

The electromagnetic spectra have been routinely used in the field of medicine to detect abnormalities and fractures and to observe healing tissues, but this worthy detection tool comes with a risk of exposing the patients to excessive radiations. Although X-rays are swift and painless, long-term exposure to their radiations could cause harmful effects including cellular damage. Many new powerful analytical tools have been developed in recent years which can deliver precise results with minimal potential damage to the body tissues. [1] Nuclear magnetic resonance (NMR) was first discovered in the 1940s. [2] The NMR uses the magnetic properties of assured atomic nuclei and is widely being used in physics and chemistry. In dentistry, this technique is predominantly beneficial to explore the structure of amorphous glasses and dental cements, bioactive glasses interaction with oral tissues, identification of salivary metabolites for disease detection, [3] [4] and understanding the periodontal diseases by gingival crevicular fluid biomarkers analysis. [5] [6] It is also commonly used to review the fluoridation of apatite surfaces in the tooth structure. [7] Therefore, this review is aimed at providing a general overview of the main principles of NMR, types of this technique, and the advantages and disadvantages of NMR spectroscopy. In addition, an insight into the current uses of NMR in the field of medicine and ongoing developments of NMR spectroscopy for future applications is discussed.

❖ Basic Mechanism of Action of NMR:

The basic principles of NMR are that the structural and chemical composition of different substances can be determined by their nuclei, which have their distinctive magnetic field. The basic NMR spectrometer analyzes using a magnetic field and a special detector to assess the changes (Fig. 1). The strength of the external magnetic field causes electrically charged nucleus to move from a lower energy level (E1) to a higher energy level (E2) and the difference between E2 and E1 is symbolized as ΔE which is dependent on the power of the magnetic field and size of the nuclear field moment.[8]

The electromagnetic radiation rhythm attains the NMR signal with a frequency (v) causing the nuclei to move to a higher energy level (E1/E2). When this electromagnetic radiation is stopped, it causes the nuclei to relax and accomplish thermal equilibrium. This release of energy from the nuclei is recorded in the form of spectra on the computer, and these spectra are exclusive for every nucleus and are equivalent to the energy levels between the two states (E2/E1) [8] (Fig. 2).

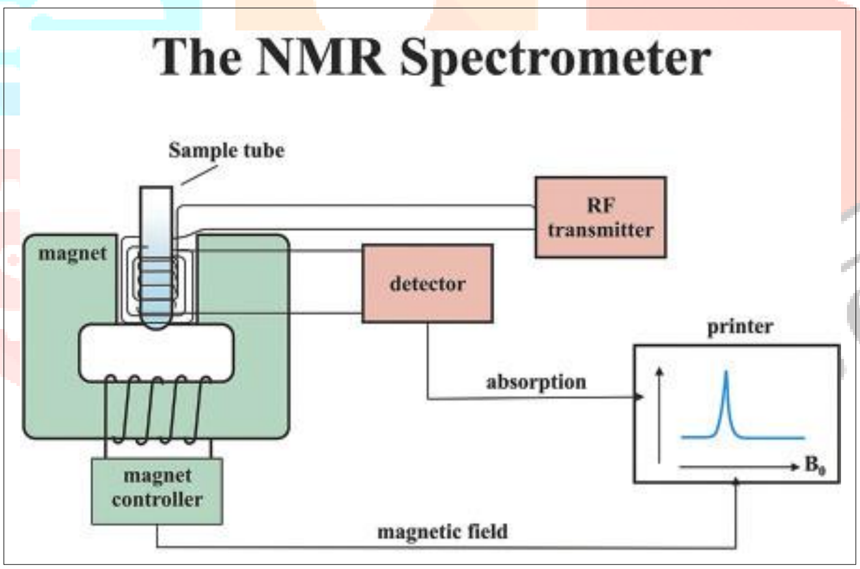

Figure 1, Schematic presentation of a typical nuclear magnetic resonance spectrometer Showing the relationship of various components (magnet, magnetic field, and detector).

➢ Magnetic Field Strength:

NMR requires a magnetic field that is both strong and uniform. The magnetic field strength is measured in Tesla or MHz. The NMR requires a reference nucleus to represent the strength of the magnetic field.

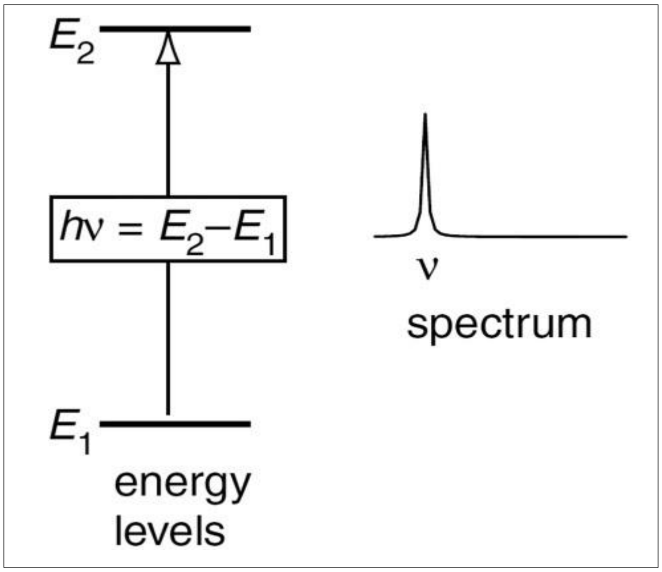

Figure 2, a line in the spectrum is related to a transition Between two energy levels (E2 and E1). [9]

➢ Chemical Shift:

The movement of the electrons creates a magnetic field in and around the nucleus. This magnetic field created is different in the direction as compared with the outer magnetic field. Any change in the magnetic field causes a similar change in the spectrum of the NMR. This sum of the shift is controlled by nature of the nucleus and nature of the motion of electrons in its surrounding atoms and molecules. This phenomenon is called "chemical shift (CS)." A reference compound is needed to measure CS [9] and to determine and differentiate magnetically inequivalent nuclei present in a molecule.

➢ Spin–Spin Coupling:

The nuclei close to each other induce an incident called spin–spin coupling (SS) due to the difference in nuclei's magnetic field direction. This direction could be either toward or opposing the magnetic field, causing the splitting of NMR signals. This magnetic field direction could either strengthen or fade the signals of NMR signals that can split into two or more components depending upon the specific nuclei having characteristic distance and relative potency. [10]

❖ TYPES OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY:

Solid in a solid state. The main technique frequently used in a solid-state NMR is magic angle spinning (MAS). This magic angle makes the resolution of the sample more precise by making the broader lines of the NMR narrower, [11] resulting in narrower signals giving isotropic values and spinning sidebands to identify the CS of the nuclei for structural determination of solid materials.

• Phosphorus Nuclear Magnetic Resonance:

In the solid-state NMR, phosphorus is one of the isotopes used to study the molecules and structures of different samples. Compound classes of phosphorus were identified which included orthophosphate diesters, polyphosphate, phosphonates, orthophosphate monoesters, and orthophosphates. [12]

- <u>Proton Nuclear Magnetic Resonance:</u>

Proton is the initial and the most frequent atom to be used in NMR spectroscopy. It is also called hydrogen-NMR (^{1}H-NMR) that provides information about the different varieties of hydrogen present in the molecule and also gives information about its adjacent surroundings.[13] ^{1}H-NMR spectrum of main materials shows small CS range for usual compound is being studied. This CS ranges from +14 to –14 ppm and a broad difference in extent of coupling constant was observed.[14]

- <u>^{27} Silicon Magic Angle Spinning Nuclear Magnetic Resonance:</u>

Silicon is an essential element, and its ^{29}Si isotope, which is used in ^{29}Si-NMR, has 4.70% natural occurrence with the half spin nucleus. It is another spectroscopic technique used to investigate the structures of organic compounds. Its value of the magnetic moment is a little low causing a low resonance frequency. The predominance of ^{29}Si-NMR shifts is present in a range from +50 to –200 ppm.[15]

- <u>^{19} Fluorine Magic angle Spinning Nuclear Magnetic Resonance:</u>

Isotopes of fluoride are naturally present in very less quantities except for the ^{19}F isotope. F-19 is the only constant isotope of fluorine found in large quantities. Due to its good nuclear qualities and a great quantity, it is used in ^{19}F MAS NMR technique. ^{19}F-NMR technique is very rapid when compared with ^{1}H-NMR technique, and without a doubt, ^{19}F nucleus is one of the most amenable NMR nuclei. Fluorine has a spin of half-nucleus. Its nucleus in molecules is usually encircled by nine electrons, and its binding energy is 147,801 keV. The sensitivity of ^{19}F-NMR spectroscopy to its CS (to study the fine details of the local surrounding) is much high for fluorine, thus making it very reactive to NMR measurements with an extremely broad CS range.[16] Yesinowski and Mobley[17] verified the capacity of this NMR to differentiate between fluorapatite, fluorohydroxyapatite, and calcium fluoride in massive phase and also on hydroxyapatite surfaces. ^{19}F-NMR can identify fluoride even in minimal concentrations starting from 0.1%. ^{19}F-NMR technique has also been used to study the metabolism of drugs containing fluoride.[18]

- <u>^{13} Carbon Nuclear Magnetic Resonance Spectroscopy:</u>

This technique is a significant tool to recognize carbon atoms in any organic material. It also gives detailed information regarding the chemical structure of the organic compound being studied. ^{13}C is an isotope of carbon which has a spin quantum number of ½ and is only 1.1% naturally present, and this isotope can be detected by ^{13}C-NMR. ^{13}C-NMR is less responsive to carbon in view of the fact that the main isotope of carbon is ^{12}C, which is not magnetically active; therefore, it cannot be detected through this technique. The intensities of the signals in carbon-NMR are not usually comparative to the number of corresponding ^{13}C atoms. They are strongly reliant on the numerals of adjacent spins.[19] Magnets utilized in C-NMR have a usual diameter of 10 mm and its usual range of CS is much larger compared with proton NMR. ^{13}C-NMR can be used to find out the composition of different molecules and is also used in the drug industry to verify drug purity.

- <u>^{27} Aluminum Magic Angle Spinning Nuclear Magnetic Resonance:</u>

^{27}Aluminum MAS-NMR has a natural abundance of 100%, and it has a 5/2 nuclear spin. The nucleus of aluminum is very responsive giving wide lines over the broad range of CS. The main application of this NMR is to identify the existence of aluminum and to observe the probable structural changes of the different varieties of aluminum. In a previous study, [27] Al-NMR has been used to observe alteration of Al (IV) into Al (VI) in the setting glass carbomer cement. [20]

❖ ADVANTAGES OF NMR SPECTROSCOPY:

1. Noninvasiveness: The fundamental quality of NMR is its noninvasiveness. Due to NMR, the studies of biological cells and tissues are now possible without damaging the sample. The fact that both spectrum and imaging can be obtained without destroying the sample is noticeably the greatest advantage of NMR as in in vivo studies. [21]
2. Lack of Ionizing Radiation: Another major advantage of NMR is its lack of ionizing radiation. Many techniques are being used in vivo studies involving ionizing radiations. NMR has made it possible to avoid the exposure from radiation, which could be harmful to both the researcher and the subject. NMR utilizes isotopes which are stable such as carbon-13 to measure metabolic fluxes instead of radioactive compounds. NMR not only minimizes the exposure to observer and subject from the harmful rays but also eliminates the need to dispose of the radioactive tissues and other materials that might be contaminated during the study. [1] Thus, the application of NMR can ensure the safety of the employees as well as reduce experimental cost due to the removal of discarded radioactive substances.
3. Adjustability: Extensive variety of processes can be investigated through NMR due to the flexibility of the particular technique that can be applied. This technique not only gives information about the physiology of the tissues, but it also gives great images of those tissues. With NMR, a single study can be performed with the same basic technique in both humans and animals, which is important to increase the translation of information. The computed tomography (CT) scan technique can only provide the imaging but not the metabolic or anatomic details, [22] whereas NMR spectroscopy has the capability of acquiring a wide variety of information.
4. Detailed Structural Analysis: Over the period, NMR has played a major responsibility in determining the mechanisms and chemical connections at a molecular level. This technique has helped to obtain information regarding the minute details about the physical and chemical characteristics of structures. [23] NMR can also analyze the parameters of CS, and it can give details on the local bonding environment around a particular atom, which could be calculated for the extended period of times with NMR. [24] It utilizes the pseudo wave function to get information about large compound structures. [25] NMR has the capability to assist studies of biochemical processes conducted in vivo, which is not efficiently achieved with other imaging techniques. Lee et al [26] proposed that NMR is a better-quality technique as compared with X-ray diffraction in determining the archaeological bone structure.

❖ DISADVANTAGES:

1. Presence of High Magnetic Field Surroundings Is Essential: An unavoidable outcome when performing the NMR technique is the requirement to perform in a surrounding which has a high magnetic field. The presence of the magnetic field can affect the proper functioning of monitors and computer-controlled devices. If any sharp objects such as a scalpel, scissors, or stapler are present in the NMR magnetic field area, it can get attracted to the magnet field which can cause severe injuries to the workers. Nowadays, monitoring devices used in the magnetic field area are being designed to function properly under the magnetic field. [27] Furthermore, instruments being used in NMR studies are made nonferromagnetic to reduce the problems encountered with high magnetic field surroundings. [28] NMR system cannot be purchased by a single investigator or for single research because of its high cost.
2. The greatest disadvantage of NMR spectroscopy and imaging compared with other modalities is the intrinsic insensitivity of the methods. The signal that can be generated in the NMR experiment is small and, for practical purposes, most strongly coupled with the concentration of the nuclei in the sample.
3. This is good for more accurate determination of the structure, but not for the availability of higher molecular weight.
4. The resolving power of NMR is less than some other type of experiments (ex. X-RAY CRYSTALLOGRAPHY) since the information got from the same material is much complex.
5. Unfortunately we are just able to determine degree of probability of being of protein segment in the confirmation.
6. The cost of the experimental implementation is increasing with the higher strength & the complexity of determination.
7. NMR experiment is small and, for practical purposes, most strongly coupled with the concentration of the nuclei in the sample

❖ APPLICATIONS OF NMR:

1) CT scan images of the cranium are restricted by artifacts, but this limitation does not occur with NMR. In the field of medicine, NMR gives the benefit of identifying pediatric tumors, hematomas, and other pathologies. [29] Since multiple sclerosis is a very tricky disease to identify, NMR has become the prime diagnostic device for multiple sclerosis. [15] NMR has particular use for certain body areas such as brain where it produces very detailed and definite images showing delineation between gray and white matter, [30] whereas some tissues such as bone, having low water percentage cannot emit strong signals to create images for NMR. [31] Moreover, NMR is apparently victorious in identifying breast cancer at an early stage. According to a radiologist at Cleveland, a mammogram cannot differentiate between small cancer and a spot, when there are multiple cysts in the breast; however, with NMR, this distinction is possible. [32] NMR technique also gives good images of fatty tissues, and a large quantity of fat creates wonderful images. In addition, the diagnosis of vascular diseases is promising with the NMR [33] as it enables the detailed structural analysis of the surfaces of blood vessels and their abnormalities.

2) By studying the peaks of nuclear magnetic resonance spectra, chemists can determine the structure of many compounds. It can be a very selective technique, distinguishing among many atoms within a molecule or collection of molecules of the same type but which differ only in terms of their local chemical environment. NMR spectroscopy is used to unambiguously identify known and novel compounds, and as such, is usually required by scientific journals for identity confirmation of synthesized new compounds.

3) Nuclear magnetic resonance is extremely useful for analyzing samples non-destructively. Radio-frequency magnetic fields easily penetrate many types of matter and anything that is not highly conductive or

inherently ferromagnetic. For example, various expensive biological samples, such as nucleic acids, including RNA and DNA, or proteins, can be studied using nuclear magnetic resonance for weeks or months before using destructive biochemical experiments. This also makes nuclear magnetic resonance a good choice for analyzing dangerous samples.

4) In dentistry, the aim of treatment is to preserve natural tissue and reconstruct the loss tissue with the help of biomaterials. These dental biomaterials are studied by many characterizing machines such as mechanical tester, physical testing, rheological testing, and biocompatibility testing. For that, NMR spectroscopy is a miracle machine to understand in-depth chemical reaction of materials ingredients and their effect with natural tissues. Extensive research on gas ionomer cement (GIC), resin composites, dental bone cements, and periodontal membranes materials has been conducted using the NMR spectroscopy. Prosser et al used NMR spectroscopy and reported the role of tartaric acid in the setting reaction of GIC, was "The fluid cement pastes have shown that tartaric acid reacts more readily than the polyacid with the glass, and hence suppresses the premature gelation of the cement." [34] A novel antimicrobial polymeric dental restorative material was experimentally synthesized to see the biocompatibility, strength, and re-mineralization property by NMR (^{1}H- and ^{13}C-NMR) spectroscopy. [35] The advancements of proteomics in dentistry have brought a revolution in the management of oral diseases and analysis of molecular changes during the reconstruction or rehabilitation of oral tissues (soft and hard) with dental materials. [36] To observe the orthodontically induced external apical root resorption biomarkers, Zhou et al studied the ^{1}H-NMR-based metabolomics and detected the inflammatory metabolites from saliva samples. [37] This study brings an importance of NMR spectroscopy in the field of clinical dentistry and dental early diagnosis.

5) All the examples mentioned above show that NMR spectroscopy in the hands of chemists , physicists and biologists is perhaps the most powerful tool for studying the matter at a molecular level. A new category of applications is the so-called NMR microscopy or NMR imaging, which is based on the same basic principles as the classical NMR. The difference here is that the signals that are produced vary according to the nuclear density and the properties of the surrounding. Therefore, it is feasible to localize in the three dimensional space the magnetic nuclei (usually protons) allowing the representation of images and the observation of crosscuts of objects with an extraordinary resolution that reaches the millionth of the meter. NMR microscopy exhibits a variety of technological applications such as the detection of microscopic defects in plastic tubes, the diamond localization in order to avoid breakings during the procedures that follow their excavation. NMR microscopy can also provide valuable information about the fruits. Ripening , the best conditions for food handling and even yet about the best cooking temperature conditions, so, unpleasant surprises with frozen fruits returning home after shopping could be avoided.

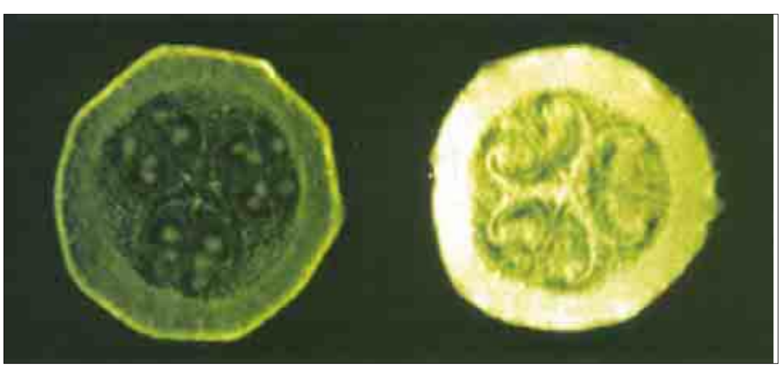

Figure 3. NMR microscopy of a fresh fruit (on the left) and of a frozen one (on the right). Note that the two images were taken without the crosscut of the fruit.

6) It was an autumnal morning of the seventies, when several hundreds of demonstrators were gathered in front of a central hospital of an American city protesting against the installation of a bulky instrument with the peculiar name: Nuclear Magnetic Resonance Tomograph. Their main demand was to install this .dangerous. Instrument away from the center of the city and of any habitable area. Furthermore, the absence of satisfactory security measures (there were only two or three signs, which were forbidding the entrance to those people carrying pacemakers) strengthened the anxiety of the demonstrators. Was this demonstration justified? Has NMR spectroscopy anything to do with the emission of dangerous radiation since it is a .nuclear. Phenomenon? The answer is no according to the basic principles, which were outlined above: NMR spectroscopy uses only radiofrequencies. Indeed, the identification of different kind of tissues as well as the visualization of organs. movement consist a very important field of medi-care applications, knowing as magnetic tomography or Magnetic Resonance Imaging, *MRI*. Medical community has been very enthusiastic with these new techniques due to many advantages compared to X-rays or to other imaging methods that use radioactive compounds. For instance, the blood volume that is conveyed by the heart in one pulse can be measured, allowing the study of the heart in action. Moreover, soft tissues that it is impossible to be detected using X-rays are clearly shown with the MRI technique, allowing the diagnosis of tumors and of other diseases. Figure 20 shows a typical application of this technique providing a diagnosis in human brain damages. Recently, investigators claimed that NMR tomography will send very soon the biopsy to retirement. It is well known that during biopsy human tissue is subtracted and the malignancy of a neoplasm is examined under the microscope. On the contrary during the NMR tomography no tissue abstraction is taking place. Instead, a chemical compound with specific properties is infused in human's blood. This compound presents different accumulation in benign tumors in relation to malignant ones. With this methodology, which is completely painless, the insight morphology between, for example, the benign and the malignant breast cancer can be distinguished.
7) It is obvious that the development of NMR spectroscopy has overcome the most optimistic predictions providing an extremely wide field of impressive applications that are beyond the field of chemistry and are extended to physics, biology, medicine and related disciplines. Therefore, it was not a surprise for the NMR community the recent discovery that NMR can be used for developing quantum computers, with counting speed that reaches million times the speed of the most recent computers. Perhaps it is not a utopia to imagine the time when every personal computer will be connected to its own NMR instrument.

Figure 4, Magnetic Resonance Imaging (MRI): Anatomical view of the brain

8) A rapid and quantitative 1H nuclear magnetic resonance (NMR) method was developed to analyse histamine in cheeses. The procedure is simple because the acid extract is analyzed directly, without any need for further filtration, derivatization, or other manipulation. The NMR method was successfully applied to different types of cheese, ranging from soft to hard [38].

9) The application of 1H nuclear magnetic resonance (NMR) spectroscopy to the measurement of conjugated linoleic acid (CLA) content in the lipid fraction of dairy products is both a novel and inviting alternative to traditional methods such as gas chromatography (GC), which can require time-consuming sample derivatization. 1H NMR analysis approach has potential application in the dairy industry as a screening technique for total CLA concentrations in large numbers of cheese samples and in the screening of CLA content in other dairy products [39].

10) Solid-state 31P nuclear magnetic resonance (NMR) to determine the different states of phosphates in cheeses was used. Sixteen semi-hard cheeses of various compositions were studied, and three fractions of phosphates (P) were distinguished according to their mobility: (1) mobile soluble P (ca. 10 % of total P), (2) mobile insoluble P (70 %) and (3) immobile insoluble P (20 %). In accordance with chemical composition and buffering capacities of the cheeses, these fractions could represent respectively (1) soluble inorganic P,(2) inorganic colloidal calcium P and phosphorylated serine residues (Pser) involved in a loose structure and (3) Pser involved in a tight environment.It was thus demonstrated that solid-state NMR is an appropriate method to observe the distribution of phosphates in cheese matrix and their evolution during cheese-making [40].

11) One-dimensional 31P NMR and two-dimensional (2D) 31P,1H COSY NMR spectroscopy was used for the determination of the phospholipids which comprise an important important lipid class in food because of their technological use as emulsifiers and their nutritional value. The total phospholipids content in cheese fat and fish oil ranged from 0.3 to 0.4% and from 5 to 12%, respectively. Minor phospholipids were identified in forms of phosphatidic acid, lysophosphatidic acid, and phosphatidylglycerol [41].

12) Discrimination between apple juices produced from different varieties has been achieved by applying principal components analysis (PCA) and linear discriminant analysis to 1H NMR spectra of the juices by Belton et al. Under optimum conditions a 100% success rate was achieved. Examination of the principal component loadings showed that the levels of malic acid and sucrose were two important chemical variables, but variations in the composition of the minor constituents were also found to make a significant contribution to the discrimination [42].

13) Green teas from different countries was collected and analyzed by 1H NMR. It was proposed to establish if the teas could be discriminated according to the country of origin or with respect to quality. After an extensive assignment of spectra, NMR spectroscopy has been shown to provide a wealth of information about the main metabolites of the teas studied. Tea components were determined for discrimination of teas as shown Figure 3 [43].

14) Application of 1H NMR was used quality control and authenticity of instant coffee by Charlton et al. The presence of inherent differences between coffees produced by different manufacturers, and even between those produced by the same manufacturer, by identifying 5-(hydroxymethyl)-2-furaldehyde as a marker compound using the structural characteristics were determined by NMR [44].

15) Another study, 31P NMR was used to determine the amount of mono- and diglycerides in virgin olive oils. It was found that quantification of other constituents of olive oils bearing functional groups with labile protons could be extended by quantitative 31P NMR spectroscopy [45].

16) NMR can be used for foodomics because of ease of quantification and identification, short time and low costs needed for analysis and high number of metabolites that can be measured through a single-pass. Because of highest sensitivity of NMR focus on hydrogen is prefered for foodomics studies [46].

17) The molecular fatty components of Pecorino Sardo Protected Designation of Origin (PS PDO) cheese were characterized through an exhaustive investigation of the 1 H- and 13C-NMR spectra of the extracted lipids. Several fatty acids (FA), such as long chain saturated, oleic, linoleic, linolenic, butyric, capric, caprylic, caproic, trans vaccenic, conjugated linoleic acid (cis9, trans11–18:2), and caproleic (9–10:1) were unambiguously detected. The positional isomery of some acyl groups in the glycerol backbone of triacylglycerols (TAG) was assessed. The NMR signals belonging to 1,3 diacylglycerols (DAG), and free fatty acids (FFA) were analysed as a measure of lipolytic processes on cheese. 1H-NMR resonances of saturated aldehydes and hydroperoxides were detected [47.]

18) Studies has shown that characterization of geographical origin could be done by using NMR spectroscopy. NMR technique was used for characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. Isotopic ratios (13C/12C and 15N/14N) and other variables were affected by the specific area of origin of milk samples while NMR data, together with isotopic ratios, were useful for the discrimination of mozzarella samples [48].

❖ CONCLUSION:

Considering the potential advantages of the NMR technique, it can be concluded with authority that it has become a preferred choice of technique for any diagnosis, treatment planning, and maintenance of treatment and also to see the behavior of foreign materials interaction with the human body. NMR is still a growing technology, and it is being anticipated that few discoveries are now just around the corner.

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