10 chapter

Nuclear Magnetic Resonance

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10.1 INTRODUCTION

Nuclear magnetic resonance (NMR) **spectroscopy** is a powerful analytical technique with a wide variety of applications. It may be used for complex structural studies, for protocol or process development, or as a simple quality assay for which structural information is important. It is nondestructive, and high-quality data may be obtained from milligram, even microgram, quantities of sample. Whereas other spectroscopy techniques may be used to determine the nature of the functional groups present in a sample, only NMR spectroscopy can provide the data necessary to determine the complete structure of a molecule. The applicability of NMR to food analysis has increased over the last three decades. In addition to improved instrumentation and much lower costs, very complex and specialized NMR techniques can now be routinely performed by a student or technician. These experiments can be set up with the click of a button/icon, as all the basic parameters are included in default experiment files listed in the data/work station software, and the results are obtained in a short time.

NMR instruments may be configured to analyze samples in solutions or in the solid state. In fact, these two types of analyses can be used in tandem to follow the fate of a given molecule within a specific system. For example, as a fruit ripens, many components will be released from the solid matrix around the plant cells into solution in the ripe fruit liquid. The development of this process can be followed by liquids versus solids analyses during the ripening time. As ripening progresses, some NMR signals will decrease in the solids NMR analyses and increase in the liquids NMR spectra.

Other food applications of NMR spectroscopy include structural analysis of food components, such as fiber, to correlate the structure to the rheological properties. Routine analyses are used to determine the quality of a product or to test the purity of ingredients. Related techniques, such as **NMR relaxometry**, can be used to assess processing operations; for example, relaxometry is used to follow the solubilization of powdered ingredients in water, to optimize processing parameters. **Magnetic resonance imaging** (MRI) is a nondestructive technique that can be used to image product quality and changes during processing and storage. For example, MRI is used to image the freezing process, with the goal of increasing shelf life. When combined with rheological analyses, sauce and paste flow in a processing system can be measured.

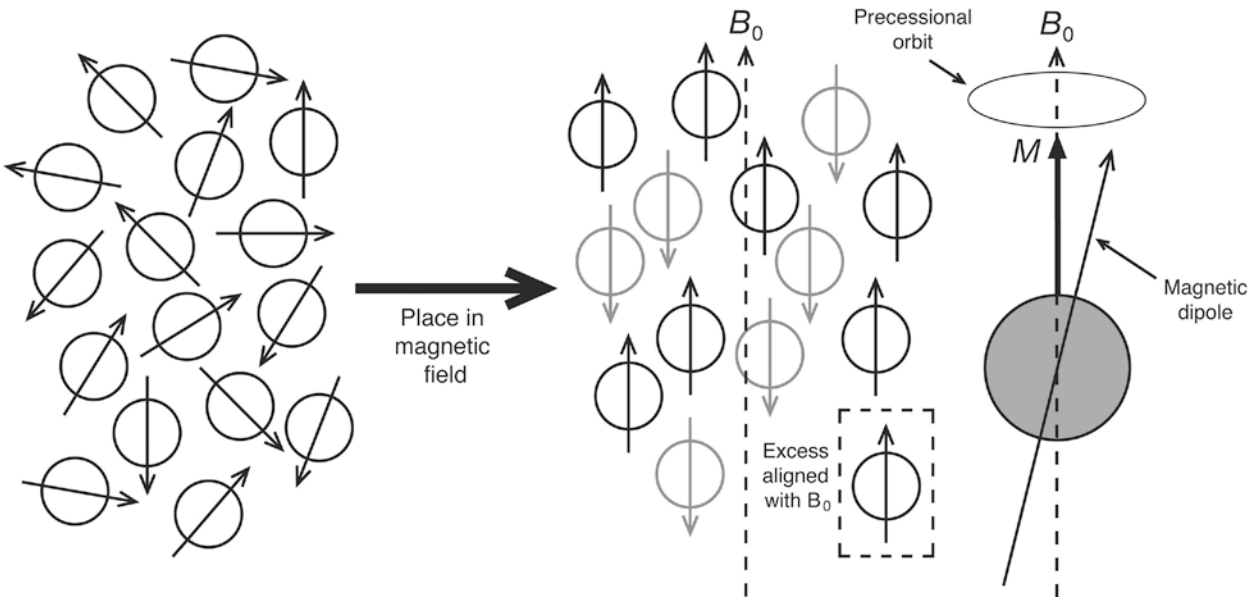
This chapter will cover the basic principles and applications of NMR spectroscopy, as well as a brief description of relaxometry, MRI, and a recent development in instrumentation that uses NMR as part of a rapid moisture and fat analysis system. Specific applications to food analysis will be highlighted.

10.2 PRINCIPLES OF NMR SPECTROSCOPY

10.2.1 Magnetic Field

NMR differs from most other forms of spectroscopy in that it is the **atomic nuclei** that are the subject of study, and the measured energy is in the radio-frequency range. Many nuclei possess an angular momentum, which means that they have a characteristic spin quantum number (I) and may be analyzed using NMR. The most common nuclei analyzed by NMR are the proton (H) and the ^{13}C isotope of carbon, as well as ^{19}F and ^{31}P, all of which have a spin $I = 1/2$. Nuclei with other spin quantum numbers will not be considered in this chapter, and the theoretical discussion will focus on the proton. These nuclei are charged, and a spinning charge generates a magnetic field. Simply put, the nuclei behave like tiny magnets that interact with an applied, external magnetic field.

Once the nuclei are placed within a strong **external magnetic field** (B_0), the spin of the nuclei will align with that field (Fig. 10.1). Because of quantum mechanical constraints (nuclei of spin I have $2I+1$ possible orientations in the external magnetic field), there are only two orientations that the spin 1/2 nuclei can adopt: either aligned with the applied magnetic field (parallel or spin +1/2) or aligned against the field (antiparallel or spin −1/2). The parallel orientation has a slightly lower energy associated with it and, therefore, has a slightly higher population. It is this excess of nuclei in the spin +1/2 state that produces the net magnetization that is manipulated and measured during an NMR experiment. The spin of the nuclei is not around the center axis but comparable to a gyration (Fig. 10.1). The motion of a spinning charged particle in an external magnetic field is similar to that of a spinning gyroscope in a gravitational field. This type of motion is known as **precession**, and there is a specific precessional orbit and frequency, the **Larmor frequency**, which is related to the magnetic properties of the nuclei. The magnitude and direction of the local magnetic field describes the magnetic moment or **magnetic dipole** of the system, and due to the precession and the lower energy state excess of nuclei, there is a net vector parallel to the applied field (Fig. 10.1).



10.1 figure

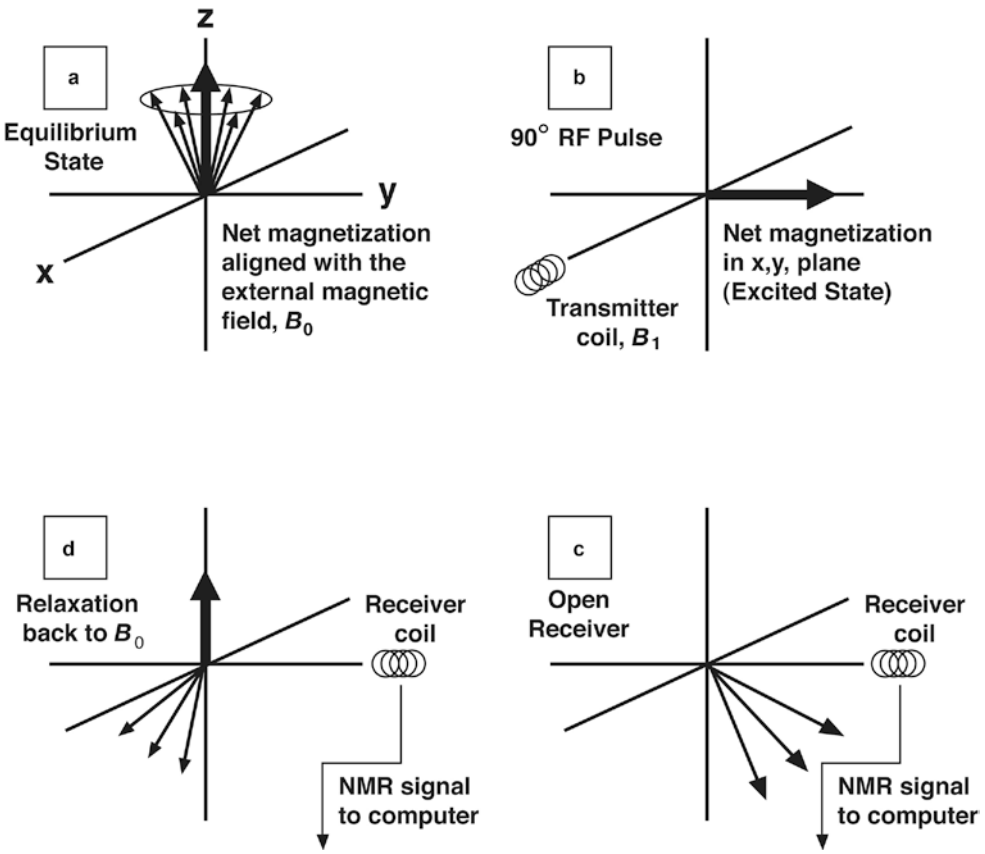
Nuclear spin and magnetic vectors are randomly ordered outside of the NMR magnet. However, once placed in an applied magnetic field, the NMR magnet and the nuclei align either with the applied field, B_0, (parallel) or against it (antiparallel). There is a slight excess in the population aligned parallel to B_0. Although the magnetic dipole tracks a precessional orbit, the net magnetization (M) is aligned with B_0

All nuclei of the same element, H, for example, will have a nearly identical Larmor frequency in a magnetic field. The specific frequency is dependent on the strength of the external magnetic field, and the Larmor frequency of H in this field defines the NMR instrument. For example, a proton has a Larmor frequency of 500 MHz in an 11.7 T magnet, so the instrument is termed a "500 MHz NMR spectrometer." The strength of the magnet not only determines the Larmor frequency of the nuclei but also the degree of excess nuclei in the parallel orientation. The excess of nuclei in the parallel orientation increases with an increase in the external magnetic field strength, and this in turn impacts the signal intensity of the NMR experiment in a higher field strength instrument. This is one reason researchers seek ever more powerful magnets for NMR (recent developments have yielded NMR magnets of greater than 23 T or 1000 MHz). Field strength impacts the signal-to-noise ratio, and, therefore, the sensitivity and resolution of the instrument and the information obtained from the NMR experiment.

The development of today's powerful NMR instruments was contingent on the advances in the production of cryo- or superconducting magnets, in which the magnet coil is held at the temperature of liquid helium (around 3 K). In addition to an increase in sensitivity, superconducting magnets also have the advantage that once charged, they maintain the magnetic field for years without the input of additional energy, due to the low temperature. The major disadvantage is the need for periodic addition of liquid N_2 and liquid He, which in the case of the latter can be quite expensive, particularly with the very large magnets associated with the high field strength instruments.

10.2.2 Radio-Frequency Pulse and Relaxation

Early NMR instruments relied on electromagnets and a simple **radio-frequency** (RF) transmitter, and the analyses were performed by a sweep through the frequency range of the instrument. The collected spectra contain the frequency information; hence, it is termed frequency-domain NMR. Although this enabled the development of NMR spectroscopy, it was not sufficient to facilitate the modern NMR experiment. One of the major developments in NMR technology was the **RF pulse**, in which a large range of frequencies is excited by a short pulse of RF energy around a centered carrier frequency, which is at the Larmor, or resonance frequency of the nuclei under study. This pulse simultaneously excites all of the protons in the sample, and the NMR data for all the protons is collected during a short time after the pulse is applied. The excitation of a range of radio frequencies by a pulse is similar to the excitation of a range of audio frequencies when a clapper strikes a bell, and the size and construction of the bell determines the range that is emitted. In NMR, the carrier



10.2 figure

(**a**) Priyor to the RF energy pulse, the net magnetization (M) composed of all the component vectors is in the equilibrium state, aligned with B_0. (**b**) The 90° RF pulse, which covers the resonance frequencies of all relevant nuclei in the sample and originates perpendicular to the z-axis (B_1), causes the nuclei to move to a higher energy state, and the net magnetization rotates into the xy-plane. (**c**) Once in the xy-plane, the net magnetic vector separates into the component vectors for each unique population of nuclei. As these oscillate in the xy-plane, they emit RF signals that are detected by the NMR instrument after passing through the receiver coil, which is located perpendicular to both B_0 and the transmitter coil. (**d**) As the component vectors continue to oscillate in the xy-plane (and emit RF signals), the nuclei begin to relax back to the equilibrium state. The NMR instrument may be set up to repeat this process, with additional pulses, numerous times; the collected data are then added together to improve the signal/noise ratio and resolution

frequency, transmitter power, and duration of the RF pulse determine the frequency range of the pulse.

Once the sample is placed in the magnet, the protons align parallel or antiparallel to the applied, external magnetic field, $\mathbf{B}_0$, with an excess in parallel orientation. The net magnetization of the nuclei in the parallel orientation is aligned with the z-axis in an xyz graphical representation of the system (Fig. 10.2a). After a pulse of RF energy is applied to the system, the nuclei precess coherently and individual nuclei absorb energy and shift to a higher energy state. The pulse, which is applied by a transmitter coil perpendicular to the z-axis ($\mathbf{B}_1$), tilts the net magnetization vector away from the z-axis and toward the **xy-plane** (Fig. 10.2b).

Although the parameters that define a pulse include the transmitter power and the pulse duration, a specific pulse used in an NMR experiment is usually described by the degree to which the net magnetization is tilted. The most common pulse is the 90° pulse, which tilts the net magnetization exactly into the xy-plane, where the receiver coil is located, thereby maximizing the resulting signals. Many NMR experiments use a series of pulses, termed a **pulse sequence**, to manipulate the magnetization. Complex pulse sequences are essential for the two-dimensional (2D) NMR experiments (Sect. 10.2.5) that are required for structural analyses of complex molecules.

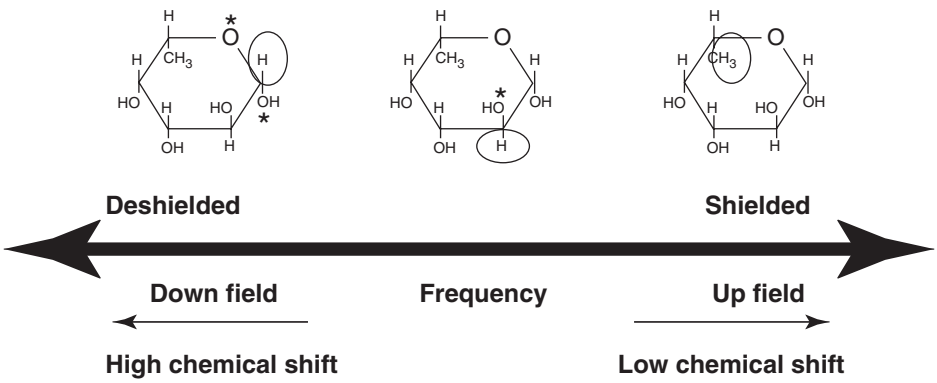
Once the net magnetization has been tilted into the xy-plane by a 90° pulse, the magnetization begins to decay back to the z-axis. This process is termed **NMR relaxation**, and it involves both **spin-lattice** ($T1$) and **spin-spin** ($T2$) relaxation. **T1 relaxation** is associated with the interaction of the magnetic fields of the excited-state nuclei with the magnetic fields of other

nuclei within the "lattice" of the total sample. **T2 relaxation** involves the interactions of neighboring nuclei that lead to a diminishment in the energy state of the excited-state nuclei and the loss of phase coherence. The mechanisms behind relaxation are complex, but the process can be utilized for some specific NMR experiments that, among other things, take advantage of the fact that samples in different forms, liquids vs. solids, for example, relax at different rates (this is very important for the instruments and experiments that are utilized for food processing and content analyses).

10.2.3 Chemical Shift and Shielding

The total H population in the sample determines the net magnetization of the system in the external magnetic field, $\mathbf{B}_0$. The exact frequency of a unique population of protons (i.e., all protons in a specific chemical configuration in the molecule), however, is also dependent on the immediate environment of the nucleus, principally the density of the electron cloud surrounding the nuclei, which determines the electronic environment of the nuclei. This is referred to as the "**shielding**" effect, because the electrons create a secondary, induced magnetic field that opposes the applied field, shielding the nuclei from the applied field. The resulting frequency differences are so small, relative to the Larmor frequency, that they are commonly reported in parts per million (ppm). However, they are large enough to be clearly detected and resolved during an NMR experiment. The frequency differences that result from the differences in the electronic environments yield the **chemical shift** of the nuclei. Following the processing of the NMR data, these differences result in a series of resonance signals, each representing a unique proton population, along the *x*-axis in a one-dimensional (1D) NMR spectrum. Those protons that have a relatively dense electron cloud are considered shielded, since the electron cloud works in opposition to the external magnetic field, and the resonances will be found on the right, or upfield, side of the spectrum, at a lower chemical shift. As deshielding increases, the resonances are shifted further to the left, or downfield, at a progressively higher chemical shift (Fig. 10.3).

One of the most important determinants of the chemical shift for a specific population of protons (i.e., all the protons in the sample that are in an identical molecular environment) is the proximity to an electronegative group or atom, such as O (Fig. 10.3). For example, protons that are not located near (in a molecular context) any electronegative groups or atoms, such as those in the methyl group of a 6-deoxy sugar, are heavily shielded, and the resonances will be found on the far right side of a proton NMR spectrum (Fig. 10.3). In contrast, the proton on the C1 of a typical sugar (the anomeric proton) is near two O atoms, the O in the –OH group (or the O in the linkage to the next sugar in a polymer, such as starch) and the O that forms part of the hemiacetal ring structure of the sugar. Consequently, the resonances associated with the highly deshielded anomeric protons are found on the left side of the proton spectrum. Protons near one O, such as the ring protons in a typical sugar, will be partially shielded, and the resonances associated with these protons will be in the central region of the spectrum.



10.3 figure

The shielding effect is responsible for the small, but detectable, differences in the resonance frequencies of nuclei such as protons. Protons that are not close to an electronegative group in the molecule, such as the protons in the methyl group in fucose (*top right*), a common 6-deoxy sugar, will be shielded by the electrons surrounding it, and it will have a low chemical shift, upfield in the NMR spectrum. Protons near one oxygen atom (indicated by an *asterisk*), such as the ring protons in sugars (*top middle*), will be intermediately shielded and have a chemical shift toward the middle of the spectrum. And a proton that is near two oxygen atoms, such as the anomeric proton in sugars (*top left*), will be relatively deshielded and have a high chemical shift downfield in the spectrum

10.2.4 1D NMR Experiment

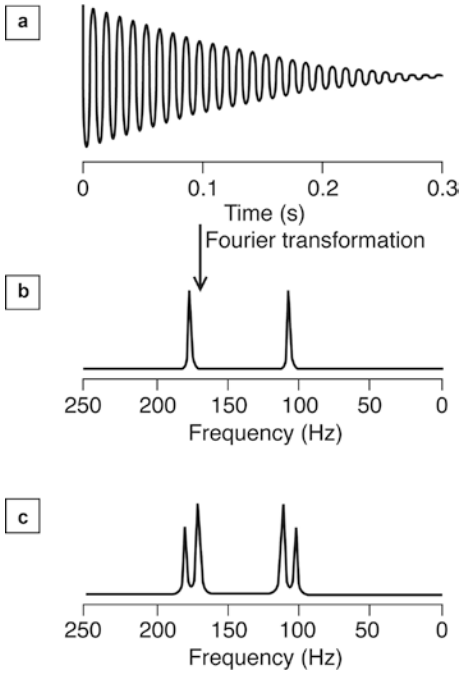
For solution ^{1}H-NMR spectroscopy, the sample is dissolved in deuterated solvents produced for NMR analysis, such as D_20 (where D = deuterium or ^{2}H; this is to avoid overloading the NMR signal with solvent protons), and pipetted into a NMR tube, which is then capped and placed into the magnet. The net magnetization of the nuclei in the parallel orientation in the external magnetic field, $\mathbf{B}_0$, is aligned with the *z*-axis; this is the equilibrium state. The 90° pulse, centered at the Larmor frequency of H, from the transmitter in the *x*-axis tilts the net magnetic vector into the *xy*-plane. There the component vectors (those representing each unique population of protons) will oscillate at their specific NMR resonance frequencies, separating the net vector into numerous component magnetic vectors, which then induce radio signals (the NMR signal) in the receiver coil located in the *y*-axis (Fig. 10.2c). At the same time, the magnitude of the vectors will decay in the *xy*-plane (relaxation) and return to the *z*-axis (Fig. 10.2d). This whole process is termed one **scan**.

The result of these actions is a combined signal for all protons in the sample, which rapidly decreases over time, yielding a **free induction decay** (FID) (Fig. 10.4a), which contains all the frequency and intensity information (as well as phase, which will not be considered) for each of the unique populations of nuclei in the sample. **Fourier transformation**, a mathematical operation that converts one function of a variable into another, is then applied to convert the time-domain FID to the frequency-domain **NMR spectrum** (an *xy* plot) (Fig. 10.4b). The frequency information for each unique proton population is presented on the *x*-axis of the NMR spectrum. The signal intensity is related to the *y*-axis; however, this is not labeled because it has no units, and, moreover, the linewidth differences of each resonance make the *y*-value imprecise for quantification in ^{1}H-NMR analyses. The best method to compare the signal intensity and, consequently, the relative abundance of specific protons in a 1D NMR spectrum is to compare the integration values of the resonances.

In practice, the NMR spectrometer is set up so that the pulse is applied numerous times, usually in increments of 16 scans. For samples that are present in a high concentration, 16 or 32 scans per experiment are common, and 256 or 512 scans are often used for more dilute samples. After each scan, the new data are added to the data already collected. The result of compiling the data from numerous scans is a significant improvement in the signal/noise ratio and resolution.

10.2.5 Coupling and 2D NMR

Another essential concept to consider is "**coupling**." Coupling is a result of the influence of electrons in covalent bonds on the local magnetic field of nearby



10.4 figures

After the 90° RF pulse moves the magnetization to a higher energy state in the *xy*-plane, the receiver is turned on, and it collects the RF signals emitted by oscillating nuclei; the emitted RF signals rapidly decay over a short time as the magnetization relaxes back to the equilibrium state (see Fig. 10.2). (**a**) The result is NMR data that contains all of the frequency and intensity information for the nuclei under analysis and which diminishes as the signals decay; this data is termed a "free induction decay" (FID), and it represents the time-domain information obtained from the NMR experiment. (**b**) Once the FID has been processed by Fourier transformation, an NMR spectrum is obtained; this represents the frequency-domain NMR information. The resonance "peaks" found on the *x*-axis are due to unique populations of protons, two in this case. (**c**) If the two protons are coupled through the molecular bonds, they will each have the effect of splitting the resonance peaks into two distinct peaks. The degree of splitting, reported in Hz, is indicative of the strength of the coupling effect

nuclei. Through the intervening bonds, two nearby nuclei will affect the chemical shift of one another, resulting in the splitting of the resonances from each unique population of nuclei into two distinct resonances (Fig. 10.4c). The coupling strength is affected by both the proximity of the nuclei to one another and the geometry of the intervening bonds. For example, protons on a carbon backbone that have a *trans* relationship ("across" from each other) have a much stron-

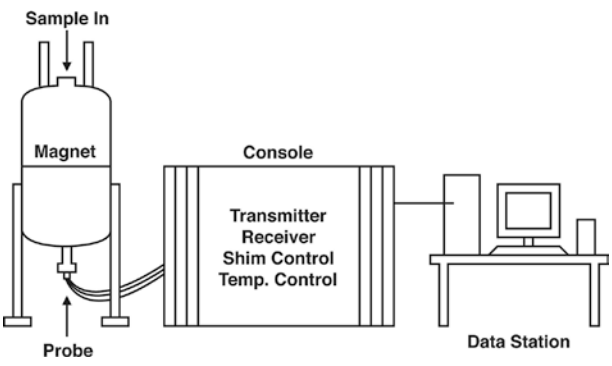
ger coupling than those that have a *cis* relationship ("on the same side" as one another). Thus, the use of coupling data yields information about the geometry of a specific molecule.

A more important impact of coupling is that complex 2D NMR experiments have been designed to take advantage of the coupling phenomenon, to produce the data necessary for the complete structure determination of a molecule. 2D NMR experiments are essentially a series of 1D experiments, in which the pulse sequence includes several pulses and a variable parameter, such as the delay time between two of the pulses. The computer collects all the spectra and plots them out as a 2D plot, in which "**cross peaks**" show the coupling correlations of nearby nuclei.

10.3 NMR SPECTROMETER

The typical research NMR spectrometer consists of a powerful cryomagnet, into which the sample is placed, a set of electronics for transmitting and collecting radio signals, and a data/work station (Fig. 10.5). Modern instruments use superconducting magnets that are cooled to a very low temperature by a jacket of liquid helium, which has a boiling point of 4.2 K. This jacket is, in turn, surrounded by an outer jacket of liquid nitrogen, which is cheaper and easier to work with than liquid helium. The core of the superconducting magnet consists of coil windings of thin wires made of superconducting alloys, such as niobium-titanium or niobium-tin. The coil (i.e., the magnet) and the coolants are contained in an insulated Dewar, termed a cryostat, which includes a vacuum chamber around the liquid jackets.

Once the magnet is cooled to the operating temperature, by the addition of the coolants, and energized by an external power supply, the magnet will maintain its charge and magnetic field for years. One of the most important aspects of maintenance for NMR instruments is the routine filling, or topping off, of the coolants. A typical research instrument is filled with liquid nitrogen on a weekly basis, and liquid helium is added monthly. Failure to maintain the coolant levels will result in the **quenching** of the magnet, in which the coolants boil off violently and the magnet loses its charge. Should this happen, the magnet will need to be refilled and recharged, at the very least, and may also require expensive repairs.



10.5 figure

A diagram of an NMR spectrometer. The instrument consists of a superconducting cryomagnet (the NMR magnet), an electronics console, and a data/work station that also controls all the functions of the instrument

Down the center of the magnet but external to the Dewar (i.e., at room temperature) is a tubular space, the **magnet bore**. A multifunction device, termed a **probe**, is placed inside the magnet bore from the bottom. Inside the probe, just inside of the main magnet coil, are small, secondary magnetic coils that receive power from the NMR instrument hardware. These small coils are manipulated by the operator to make fine adjustments to the magnetic field, to optimize the magnetic field; hence, they are called shims (in construction, a shim is a small piece of wood or metal that is placed between two layers of building material to obtain a better fit, such as when leveling a door frame). The probe also contains the coils for transmitting and receiving the RF energy. Thus, the probe is the central piece of hardware for the NMR experiment. Finally, at the top of the bore, and at the top of the whole system, is the sample insertion point. The sample tube, which is in a holder, is lowered down through the bore by a diminishing stream of forced air until it gently comes to rest at the top of the probe; there the sample is correctly aligned with the magnetic field and the probe hardware. One of the most common mistakes for an inexperienced operator is to drop the sample holder and sample into the magnet bore without the airstream flowing, which results in a rapid descent of the sample holder and sample, breaking the NMR tube and damaging the probe (as well as angering the instrument shop manager).

Connected to the magnet probe by several cables is an electronics console that includes the transmitter, the receiver, and other systems that control the NMR instrument, such as the sample temperature control unit. The transmitter includes systems to produce the pulse at the correct frequency for each nucleus that may be observed. For example, a 500-MHz NMR spectrometer requires a transmitter at 500 MHz for ^{1}H-NMR analyses and a second transmitter system at 125 MHz for ^{13}C-NMR. The console also houses the receiver electronics, which process the NMR signal from the probe receiver coils, the electronics that control the shim electromagnets, and the probe temperature control system. All of this is managed by a computer data/work station and some NMR-specific peripherals.

In contrast to the large (and expensive) research instruments are the benchtop **time-domain (TD)**

NMR systems, also termed **low-resolution NMR**. They do not provide frequency, or structural, information, but they can be applied to various types of content analyses. They are similar in size to a benchtop centrifuge and utilize a common magnet, with no need for coolants or a large Dewar flask. These instruments are relatively inexpensive and simple to use, and they have numerous food analysis applications, such as fat content.

10.4 APPLICATIONS

Table 10.1 lists recent applications of the NMR and related techniques to food analysis. The following discussion describes general applications and some specific applications to food research. See the references in Table 10.1 for detailed information on the various techniques.

10.4.1 NMR Techniques and General Applications

10.4.1.1 *Liquids*

Liquids NMR is used for relatively pure samples that readily go into solution in any of the many deuterated solvents produced for NMR analyses. Common samples include carbohydrates, proteins, lipids, phenolics, and many other classes of organic compounds. The experiment described in Sect. 10.2.4 is an example of a typical liquid 1D ^{1}H-NMR spectroscopy analysis. The result is a 1D NMR spectrum with the resonances plotted along the *x*-axis (the only plotted axis, hence, 1D). This is the simplest application of NMR spectroscopy, but it can be very informative. For example, plant- and yeast-derived β-glucans are currently an important topic of research, as they have many health-related benefits, and they are often discarded as waste from many food processing systems. NMR spectroscopy is the best analytical tool available to determine the

10.1 table Recent food applications of magnetic resonance spectroscopy and related techniques

NMR method	*Food*	*Analysis*	*Ref.*
MRI	Whole wheat bread	Water migration between arabinoxylan and gluten	[1]
^{1}H-MRI	Avocado	Nondestructive assessment of bruising	[2]
^{1}H-MRI	Honey	Authenticity screening	[3]
HR ^{1}H NMR	Pork meat	Quantitative fatty acid chain composition	[4]
qHNMR	Processed foods	Quantification of benzoic acid	[5]
^{1}H and spin-spin relaxation	Rice and potato starches	Impact of hydration levels on starch gelatinization	[6]
2D NMR	Green coffee bean extract	Analysis of organic compounds	[7]
^{1}H NMR, TOCSY, HSQC and HMBC	Hazelnut	Metabolic profiling of hazelnut cultivars	[8]
DOSY-NMR	Beverages	Sucrose quantification	[9]
^{1}H, 1H-DPFGSE, and F2-DPFGSE band-selective HSQC	Olive oil	Detection of aldehydes	[10]
^{13}C qNMR	Wine	In situ determination of fructose isomer concentration	[11]
Solid-state ^{13}C NMR	Milk protein concentrate	Change in molecular structure and dynamics of protein	[12]
UF iSQC NMR	Viscous liquid foods	Sugar content, quality testing, and determination of adulteration	[13]
TD-NMR	Mayonnaise and salad dressing	Through-package fat determination	[14]
TD-NMR	Biscuit dough	Influence of fiber on proton mobility	[15]
TD-NMR	Beef	Meat quality parameters	[16]
TD-NMR (SMART Trac™)	Organogels in cream cheese	Fat content	[17]
HR-MAS-NMR	Tomato	Metabolic profiling, tissue differentiation, and fruit ripening	[18]
^{1}H HR-MAS	Fish	Rapid assessment of freshness and quality	[19]
CP-MAS-NMR	Wheat bran	Hydration, plasticization, and disulfide bonds	[20]
CP-MAS-NMR	Starch	Chemicophysical properties	[20]

NMR nuclear magnetic resonance spectroscopy, *MRI* magnetic resonance imaging, *CP-MAS* cross polarization magic-angle-spinning NMR, *HR-MAS* high-resolution magic-angle spinning, *DOSY-NMR* diffusion-ordered ^{1}H NMR, *qHNMR*, quantitative proton NMR, *TD-NMR* time-domain NMR, *DPFGSE* double pulsed field gradient spin echo, *UF iSQC NMR* ultrafast intermolecular single-quantum coherence

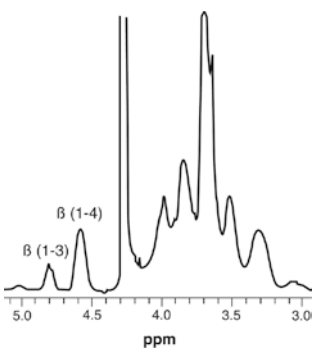
purity and identity of the β-glucans as various food processors, particularly the cereal, baking, and brewing industries, work to extract these valuable byproducts from the waste stream in a cost-effective manner. Figure 10.6 shows a 1D NMR spectrum of 1,3–1,4 mixed linkage β-glucans from cereal (oat) processing waste. From this spectrum, both the purity and relative ratio of 1,3–1,4 linkages could be determined.

If additional structural information is needed, there are many powerful 2D and 3D NMR analyses available for the assignment of the chemical shifts of each ^{1}H and ^{13}C atom in an organic molecule. Once assigned, other experiments enable an assessment of the relative proximity of these nuclei through molecular bonds and through space. 2D NMR, therefore, can be applied to any sample for which structural information is required, such as a health-related fiber or a new sweetener. This information may be critical if a company or researcher wishes to file for a patent.

NMR spectroscopy also is a valuable assay tool in batch ingredient analysis for quality assurance. In such assays, the structural assignments of the spectra would not be as important as the consistency of the spectra compared to a spectrum of a high-quality control product. This application can be used with many types of ingredients, because NMR solvents are available for compounds with a range of solubility properties.

10.4.1.2 *Solids*

The principles that underlie solid-state NMR are similar to those discussed in Sect. 10.2; however, due to the fact that the sample is not freely tumbling about in solution, there is a "directional" aspect (anisotropic or orientation-dependent interactions) to the solid-state analysis. The anisotropic nature of solids results in very broad signals and yields spectra that lack the structural information obtained from samples in solution. One method to overcome this problem is **magic-angle spinning** (MAS), in which the line broadening due to the anisotropy is countered when the sample holder is spun at a specific "magic" angle relative to the external field, B_0, yielding much narrower lines. MAS is often combined with cross polarization enhancement (CP-MAS), in which the magnetization from more easily detected nuclei is transferred to those that are less easily detected (such as from ^{1}H to ^{13}C).



10.6 figure

A 1D H-NMR spectrum of 1,3–1,4 mixed linkage β-glucans from oat processing waste. Both the purity of the sample and the ratio of 1,3–1,4 linkages could be determined from the spectrum

Solid-state NMR analyses can be applied to many types of samples, such as powders and fresh vegetable tissue. Solid-state ^{13}C-CPMAS-NMR techniques can be used to monitor the chemical composition and the physicochemical properties in the solid portion of an intact food sample. This has been applied to composition studies of different mushroom species, and solid-state ^{13}C-CPMAS-NMR spectroscopy showed significant differences in the ratio of carbohydrate to protein resonances between different species. Also, high-resolution ^{1}H-MAS-NMR techniques enabled food researchers to discriminate between durum wheat flours from Southern Italy, which differ in composition depending on the region of origin. A similar application was used to correlate composition with origin in a study of Parmesan cheese.

10.4.1.3 *Magnetic Resonance Imaging*

Magnetic resonance imaging (MRI) is unique in that the sample can be placed into the magnet in the native form, and 2D or 3D images of the sample can be generated. MRI involves variations in field strength and the center frequency of the pulses over time and space, along with the application of field gradients in different geometric positions relative to the magnet bore (B_0). The end result is a spatial "encoding" of the sample protons with different phase and frequency values. After multidimensional Fourier transformations of multiple FIDs from different spatial "slices" of the sample, an image of the sample is produced that contains information about the state of the tissue or other material under study.

The sample can be a medical patient, a small test animal, a diseased plant stem, a ripened fruit, or even a complex food product in various steps of processing or its final form. For example, a packaged product could be analyzed over time in the package to track water movement or loss. The MRI analyses would not affect the product. There are many potential applications for MRI in the food industry; for example, it can be used to image the freezing process in frozen food production, with the goal of increasing shelf life. MRI also may be applied to analyses of the composition and characteristics of pastes and sauces, to locate voids in products, or to examine the fat distribution in meats or the water/fat distribution in emulsions. It can also provide detailed information about the thickness of a

filling or coating; structural changes, including water loss, in a product via heat transfer (cooking); or changes associated with hydration of a food product during processing. When combined with rheological analyses, sauce and paste flow in a processing system may be monitored.

MRI images of clementine fruit are shown in Fig. 10.7. One image shows freeze damage to the interior pericarp region of the fruit. The other image shows the presence of an unwanted seed. Such problems often are undetected by a simple visual inspection of the fruit.

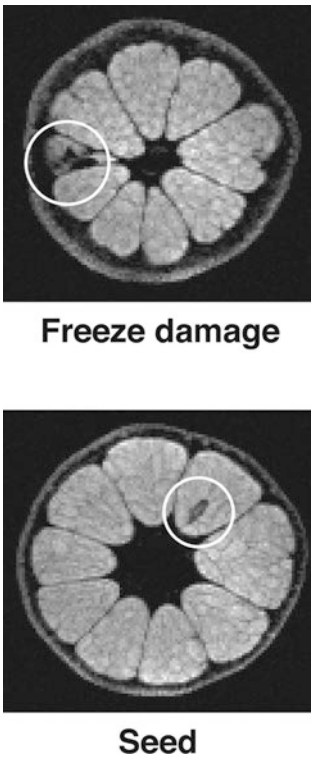
As the high costs of purchasing and maintaining a large-bore MRI instrument decrease over time, as they did for NMR spectroscopy, and as smaller bore instruments become more common, this important tool should become available to even small food companies and food science departments. These instruments may become a common sight in even modest research and development laboratories over the next decade.

10.4.1.4 *Relaxometry*

In the plastics industry, small molecules are mixed with the large polymers to make the system more fluid. These small molecules are termed plasticizers, and in food processing, the natural equivalent is water. The amount of water available to act as a plasticizer is a very important factor for food quality. An increase or a reduction in the amount of plasticizer can affect the glass transition process that, in turn, affects the quality of the final product. Water exists in several states in food, and the interaction of water molecules with food components can be investigated by the measurement of NMR relaxation. This includes both the spin-lattice ($T1$) and spin-spin ($T2$) relaxation times (Sect. 10.2.2) of the water protons. The relaxation times are related to the magnetic interactions of water protons with the surrounding environment, and the effective relaxation time is related to the extent of the association between the water molecules and immobilized or slowly moving macromolecules. In general, as the macromolecular content increases, the relaxation times of the water protons also increase.

10.4.1.5 *TD-NMR for Content Analyses*

A recent development in NMR spectroscopy is instrumentation consistent with AOAC Method 2008.06 (moisture and fat in meats), using the FAST Trac NMR system (CEM Corporation), which combines rapid drying (microwave oven) with low-resolution (time-domain) NMR in a benchtop instrument for moisture and fat analysis. The CEM Corporation (Matthew, NC) makes two such instruments that combine a microwave plus NMR for moisture/solids and fat contents (SMART TracII™ for wet products and HYBRID Trac™ for all products) and another instrument that only uses only an NMR to measure moisture /solids and fat for dry products (FAST Trac™). These relatively inexpensive systems are operator friendly and easy to maintain. They provide rapid, reproducible information for quality control of food products, yielding information on the moisture and fat content of specific food items (e.g., FAST Trac™ for chocolate and potato chips).



10.7 figure

MRI images (18 mm slice thickness) of clementine citrus fruit with defects. Freeze damage is shown in the image on the top, and an unwanted seed is shown in the image on the bottom (Images courtesy of Michael McCarthy, Aspect AI Ltd., Netanya, Israel)

10.4.2 Specific Food Application Examples

High-resolution NMR spectroscopy has been used for the analysis of complex systems such as food samples, biofluids, and biological tissues because it provides information on a wide range of compounds found in the food matrix in a single experiment. NMR spectrometry is nondestructive and offers advantages in the simplicity of sample preparation and rapidity of analysis. The short time frame needed to obtain NMR spectra (minutes), coupled with automation, enables the analysis of many samples with minimal operator input. There are two basic types of analysis in the application of NMR to the food industry: (1) identification of distinct resonances and, therefore, specific compounds and (2) use of chemometric profile analysis, in which spectral profiles are compared without assigning particular resonances.

10.4.2.1 *Oil/Fat*

10.4.2.1.1 Fatty Acid Profile

Physical and chemical properties of fats, oils, and their derivatives are mainly influenced by their fatty acid profile. Even though gas chromatography (GC) is usually used for determining the fatty acid profile (Chap. 17, Sect. 17.2.7; Chap. 23, Sect. 23.6.2), the common unsaturated fatty acids, such as oleic, linoleic, and linolenic acids in an oil or fat sample can be quantified using ^{1}H-NMR, by integration of select signals in the spectra. Although GC provides accurate information about complete fatty acid profile, it lacks information about the fatty acid distribution on the glycerol anchors, which is important to determine the functionality of the ingredient in processing, such as the crystallization point or how it plasticizes the dough in a baked product. For example, the correct type of fat is essential for quality pie crusts or croissants. The fatty acid distribution on the glycerol anchors can be obtained from ^{13}C-NMR analysis. There are two groups of resonances in the carbonyl region of the spectrum: the first is due to fatty acids in positions 1 and 3 and the second is from fatty acids in position 2 of the glycerol moiety.

10.4.2.1.2 Verification of Vegetable Oil Identity

Even though different oils or fats may be purposely mixed for specific reasons, the adulteration of high-value oils with oils of lesser value is an issue of economic and commercial importance. This is primarily a problem with olive oil, because it is expensive and has superior nutritional value. Accordingly, many studies from major olive oil-producing Mediterranean countries, such as Greece, Italy, and Spain, deal with identifying lower-value oils, such as hazelnut oil, used for adulterating olive oil. The adulteration problem is complicated by the fact that the lower-value oils usually have fatty acid profiles similar to olive oil. Among the methods used for analyzing potentially adulterated olive oil are ^{13}C-NMR and ^{1}H-NMR spectrometry. For example, NMR is utilized in conjunction with multivariate statistical analyses of specific resonances in NMR spectra of olive oil diluted with hazelnut or sunflower oil. These methods also can be used to identify the variety and geographical origin of the oil.

10.4.2.1.3 Monitoring of Oxidation

The oxidation of vegetable oils is a significant quality problem and can lead to further deterioration of the oil. Highly unsaturated fatty acids, with *bis*-allylic methylene groups, are particularly susceptible to oxidation. Primary and secondary oxidation products, such as hydroperoxides and aldehydes, are easily detected by ^{1}H-NMR analyses. ^{1}H-NMR is especially useful for such analyses because the samples do not require any additional treatments, such as derivatization, that could cause degradation.

10.4.2.1.4 Solid Fat Content (SFC)

While most analyses discussed in this chapter depend on high-resolution NMR instruments, a benchtop, low-resolution-pulsed NMR instrument can be used to determine the SFC of a sample (see also Chap. 23, Sect. 23.43.11). For example, the amount of solid triacylglycerols in the oil or fat at different temperatures can be determined. This method is based on the difference in relaxation times between solids and liquids, and after a delay, only the NMR signal of the liquid fat is measured. The solid content is then estimated. Crystallization mechanisms of fat blends also can be studied using SFC measurements.

10.4.2.2 *Water*

Glass transition is an important property of foods, and the glass transition temperature (Tg), which is dependent on water content, impacts both the processing and the storage of food products. Tg can be determined with an **NMR state diagram**, which is a curve relating NMR relaxation time to glass transition temperature at different moisture contents. This information is important because processing and storage temperatures above Tg at any point during production and distribution of a product are associated with more rapid deterioration. Spin-spin relaxation time (T2) is commonly used as an indication of proton mobility, which is different above and below the Tg of a given product. Although the differential scanning calorimeter (DSC) (Chap. 33, Sect. 33.3.2) is most commonly used for simple Tg analyses, the ability to generate NMR state diagrams increases the value of NMR for many applications.

10.4.2.3 *Ingredient Assays*

Adulteration in fruit juice is not easy to detect by taste or color. For example, orange juice can be blended with relatively inexpensive grapefruit juice, but the presence of the grapefruit juice in a commercially available orange juice product poses serious health risks for consumers with certain medical conditions. Grapefruit juice has a number of coumarin-like flavonoids and other powerful CYP450 inhibitors that negatively impact the metabolism of many prescribed drugs. Therefore, the detection and prevention of this kind of adulteration are especially important. NMR-based chemometric approaches using independent component analysis, a variant of principle component analysis, are now applied to this problem. Selected regions of the ^{1}H NMR spectra, which are known to contain distinguishing flavonoid glycoside signals, are accurately analyzed in a relatively short time. Another common issue with juice preparation is the differentia-

tion between freshly squeezed juices and those produced from pulp washes, which can be added to fresh-squeezed orange juice to reduce production costs. ^{1}H NMR, in combination with principal component analyses, can easily and accurately distinguish the fresh-squeezed and pulp-wash orange juice.

NMR is also used in monitoring batch-to-batch quality and production site differences in beer. Large multinational breweries prepare their beers at many different geographic locations and require methods for quality control at a detailed molecular level. NMR can be used in conjunction with principal component analysis to distinguish beer from different production sites based on lactic acid, pyruvic acid, dextran, adenosine, inosine, uridine, tyrosine, and 2-phenylethanol content. Quantifying these compounds allows the producers to identify production sites where there is greater variability in these compounds (and therefore poorer quality control).

NMR methods are used by other producers to improve quality control in soft drink production, juice production, and vegetable oil manufacturing. Similar methods also are used to monitor the quality of functional foods and nutraceuticals (food extracts with positive medicinal effects) that are harvested from different geographic locations.

10.5 SUMMARY

Nuclear magnetic resonance technology provides powerful research instrumentation for a variety of applications, from structural elucidation of complex molecules, to 3D-imaging of fresh tissue, to simple ingredient assays for quality assurance. NMR differs from most other forms of spectroscopy because the nucleus is the subject of analysis, and the excitation step uses radio-frequency electromagnetic energy. The proton (H) and the ^{13}C isotope are the most commonly studied nuclei, and each has a characteristic charge and spin which results in a small, local magnetic field. NMR analyses require an external magnetic field, which causes the local magnetic fields of the nuclei to align in a parallel or antiparallel orientation. There is a slight excess in the parallel orientation (in the *z*-axis aligned with B_0), and it is the net magnetic vector of this population that is detected during an NMR experiment. A pulse of RF energy moves this net magnetism into the *xy*-plane, where a reemitted radio signal (the NMR signal) is detected. This signal, which decays quickly, contains the intensity and frequency information for all the nuclei in the sample, and the resulting FID is converted by Fourier transformation into the NMR spectrum, which shows the various resonances spread along the *x*-axis based on differences in frequency.

The NMR instrument consists of a cryomagnet with the transmitter and receiver antennae in the central bore, an electronics console with the transmitter and receiver hardware, and a data/work station that controls all the functions of the instrument. In addition to NMR spectrometers, with both solids and liquids applications, there are other related instruments, such as MRI, that are based on the same principles, but yield different information.

Among the common applications of NMR to food science are structural studies that examine the correlation between chemical structure and health benefits or functionality of food ingredients, studies of the effects of processing on food properties and quality, composition studies of food ingredients or even fresh vegetable tissue, imaging of food products, and determination of SFC or ingredient purity.

10.6 STUDY QUESTIONS

1. Explain the basic principles associated with NMR spectroscopy, including the function of the magnet and the concept of nuclear spin.
2. Describe the interaction of the net magnetization with the RF pulse (90°) and the subsequent NMR signals.
3. Explain the concept of shielding and chemical shift.
4. Describe the FID and the NMR spectrum, including the concepts of time domain, frequency domain, and data transformation.
5. List the components of the NMR spectrometer and their functions.
6. What kinds of samples are analyzed by (a) liquid NMR and (b) solid-state NMR?
7. What kind of final data does one obtain with an MRI? List two applications of MRI.
8. What is the primary use of relaxometry in food analysis?
9. List the general types of food applications of NMR and give an example of each.

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