23

chapter

Infrared Spectroscopy

Randy L. Wehling

Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68583-0919, USA
rwehling1@unl.edu

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S.S. Nielsen, *Food Analysis*, Food Science Texts Series, DOI 10.1007/978-1-4419-1478-1_23,

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23.1 INTRODUCTION

Infrared (IR) spectroscopy refers to measurement of the absorption of different frequencies of IR radiation by foods or other solids, liquids, or gases. IR spectroscopy began in 1800 with an experiment by Herschel. When he used a prism to create a spectrum from white light and placed a thermometer at a point just beyond the red region of the spectrum, he noted an increase in temperature. This was the first observation of the effects of IR radiation. By the 1940s, IR spectroscopy had become an important tool used by chemists to identify functional groups in organic compounds. In the 1970s, commercial near-IR reflectance instruments were introduced that provided rapid quantitative determinations of moisture, protein, and fat in cereal grains and other foods. Today, IR spectroscopy is used widely in the food industry for both qualitative and quantitative analysis of ingredients and finished foods.

In this chapter, the techniques of mid- and near-IR spectroscopy are described, including the principles by which molecules absorb IR radiation, the components and configuration of commercial IR spectrometers, sampling methods for IR spectroscopy, and qualitative and quantitative applications of these techniques to food analysis.

23.2 PRINCIPLES OF IR SPECTROSCOPY

23.2.1 The IR Region of the Electromagnetic Spectrum

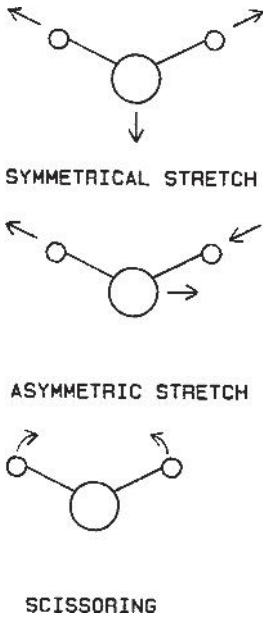
Infrared radiation is electromagnetic energy with **wavelengths** (λ) longer than visible light but shorter than microwaves. Generally, wavelengths from 0.8 to 100 micrometers (μm) can be used for IR spectroscopy and are divided into the near-IR (0.8–2.5 μm), the mid-IR (2.5–15 μm), and the far-IR (15–100 μm) regions. One μm is equal to 1×10^{-6} m. The near- and mid-IR regions of the spectrum are most useful for quantitative and qualitative analysis of foods.

IR radiation also can be measured in terms of its **frequency**, which is useful because frequency is directly related to the energy of the radiation by the following relationship:

$$E = h\nu \qquad [1]$$

where:

E = energy of the system
h = Planck's constant
ν = frequency, in hertz



23-1 figure Vibrational modes of the water molecule. Frequencies of the fundamental vibration for the symmetrical stretch, asymmetric stretch, and scissoring motion are 3652 cm^{-1}, 3756 cm^{-1}, and 1596 cm^{-1}, respectively.

Frequencies are commonly expressed as **wavenumbers** ($\bar{\nu}$, in reciprocal centimeters, cm^{-1}). Wavenumbers are calculated as follows:

$$\bar{\nu} = 1/(\lambda \text{ in cm}) = 10^4/(\lambda \text{ in } \mu\text{m}) \qquad [2]$$

23.2.2 Molecular Vibrations

A molecule can absorb IR radiation if it vibrates in such a way that its charge distribution, and therefore its electric dipole moment, changes during the vibration. Although there are many possible vibrations in a polyatomic molecule, the most important vibrations that produce a change in dipole moment are stretching and bending (scissoring, rocking, twisting, wagging) motions. Examples of these vibrations for the water molecule are shown in Fig. 23-1. Note that the stretching motions vibrate at higher frequencies than the scissoring motion. Also, asymmetric stretches are more likely to result in a change in dipole moment, with corresponding absorption of IR radiation, than are symmetric stretches.

23.2.3 Factors Affecting the Frequency of Vibration

A molecular vibration can be thought of as a harmonic oscillator, with the energy level for any molecular vibration given by the following equation:

$$E = (v + 1/2)\,(h/2\,\pi)\sqrt{k/\frac{m_1 m_2}{m_1 + m_2}} \qquad [3]$$

where:

v = vibrational quantum number (positive integer values, including zero, only)
h = Planck's constant
k = force constant of the bond
m_1 and m_2 = masses of the individual atoms involved in the vibration

Note that the vibrational energy, and therefore the frequency of vibration, is directly proportional to the strength of the bond and inversely proportional to the mass of the molecular system. Thus, different chemical functional groups will vibrate at different frequencies. A vibrating molecular functional group can absorb radiant energy to move from the lowest ($v = 0$) vibrational state to the first excited ($v = 1$) state, and the frequency of radiation that will make this occur is identical to the initial frequency of vibration of the bond. This frequency is referred to as the **fundamental absorption**. Molecules also can absorb radiation to move to a higher ($v = 2$ or 3) excited state, such that the frequency of the radiation absorbed is two or three times that of the fundamental frequency. These absorptions are referred to as **overtones**, and the intensities of these absorptions are much lower than the fundamental since these transitions are less favored. Combination bands can also occur if two or more different vibrations interact to give bands that are sums of their fundamental frequencies. The overall result is that each functional group within the molecule absorbs IR radiation in distinct wavelength bands rather than as a continuum.

23.3 MID-IR SPECTROSCOPY

Mid-IR spectroscopy measures a sample's ability to absorb light in the 2.5–15 µm (4000–650 cm^{-1}) region. Fundamental absorptions are primarily observed in this spectral region.

23.3.1 Instrumentation

Two types of spectrometers are used for mid-IR spectroscopy: dispersive instruments and Fourier transform (FT) instruments. Almost all newer instruments are of the FT type.

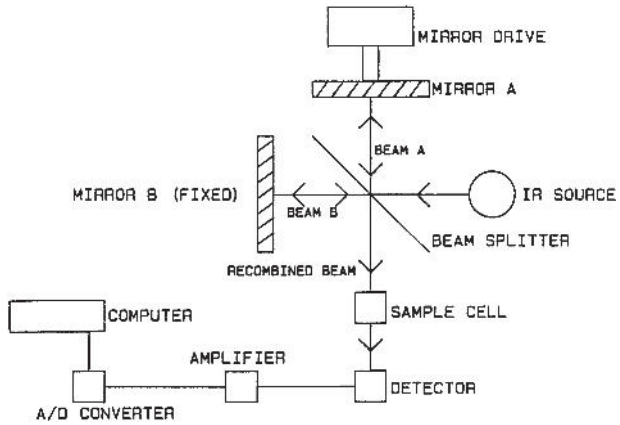
23.3.1.1 Dispersive Instruments

Dispersive instruments use a **monochromator** to disperse the individual frequencies of radiation and sequentially pass them through the sample so that the absorption of each frequency can be measured. IR spectrometers have components similar to ultraviolet-visible (UV-Vis) spectrometers, including a radiation source, a monochromator, a sample holder, and a detector connected to an amplifier system for recording the spectra. Most dispersive IR spectrometers are double-beam instruments.

A common mid-IR source is a coil of **Nichrome wire** wrapped around a ceramic core that glows when an electrical current is passed through it. A **Globar**, which is a silicon carbide rod across which a voltage is applied, also can be used as a more intense source. Older spectrometers used sodium chloride prisms to disperse the radiation into monochromatic components, but more modern instruments use a diffraction grating to achieve this effect. Detectors include the **thermocouple detector**, whose output voltage varies with temperature changes caused by varying levels of radiation striking the detector, and the **Golay detector**, in which radiation striking a sealed tube of xenon gas warms the gas and causes pressure changes within the tube. However, most current instruments use either **pyroelectric** detectors, such as deuterated triglycine sulfate (DTGS) crystals, or solid-state **semiconductor detectors**. Variation in the amount of radiation striking a DTGS detector causes the temperature of the detector to change, which results in a change in the dielectric constant of the DTGS element. The resulting change in capacitance is measured. Semiconductor detectors, such as those made from a mercury:cadmium:telluride (MCT) alloy, have conductivities that vary according to the amount of radiation striking the detector surface. These detectors respond faster and to smaller changes in radiation intensity than other detectors; however, they typically require cryogenic cooling. DTGS and MCT detectors are the most commonly used detectors in Fourier Transform instruments discussed in Sect. 23.3.1.2.

23.3.1.2 Fourier Transform Instruments

In **Fourier transform** (FT) instruments, the radiation is not dispersed, but rather all wavelengths arrive at the detector simultaneously and a mathematical treatment, called an **FT**, is used to convert the results into a typical IR spectrum. Instead of a monochromator, the instrument uses an **interferometer**. In a Michelson interferometer, which is the most commonly used design, an IR beam is split and then recombined by reflecting back the split beams with mirrors (Fig. 23-2). If the pathlength of one beam is varied by moving its mirror, the two beams will interfere either constructively or destructively as they are combined, depending on their phase difference. Therefore, the intensity of the radiation reaching the detector varies as a function of the optical path difference, and the



23-2 figure Block diagram of an interferometer and associated electronics typically used in an FTIR instrument.

pattern of energy intensity obtained as a function of optical path difference is referred to as an **interferogram**. When a sample is placed in the recombined beam ahead of the detector, the molecules in the sample absorb at their characteristic frequencies, and thus the radiation reaching the detector is modified by the presence of the sample. This interferogram showing intensity vs. pathlength is then converted by Fourier transformation into an IR spectrum giving absorbance vs. frequency. A computer allows the mathematical transformation to be completed rapidly. Because all wavelengths are measured at once, FT instruments can acquire spectra more rapidly, with a greatly improved signal-to-noise ratio, as compared to dispersive instruments.

23.3.1.3 Sample Handling Techniques

Liquids may be measured by **transmission IR spectroscopy**. Because absorptivity coefficients in the mid-IR are high, cells with pathlengths of only 0.01–1.0 mm are commonly used. Quartz and glass absorb in the mid-IR region, so cell windows are made of non-absorbing materials such as halide or sulfide salts. Halide salts are soluble in water, and care must be taken when selecting cells for use with aqueous samples. Cells are also available with windows made from more durable and less soluble materials, such as zinc selenide, but are more expensive than those with halide salt windows. Transmission spectra of solids can be obtained by finely grinding a small amount of the sample with potassium bromide, pressing the mixture into a pellet under high pressure, and inserting the pellet into the IR beam. An alternative technique is to disperse a finely divided solid in Nujol mineral oil to form a mull.

Attenuated total reflectance (ATR) **cells** are available for obtaining spectra from solid samples that are too thick for transmission measurements, pastes such as peanut butter, and liquids including viscous liquids. ATR measures the total amount of energy reflected from the surface of a sample in contact with an IR transmitting crystal. IR radiation passes through the crystal to the sample, where the radiation penetrates a short distance into the sample before it is reflected back into the transmitting crystal. Therefore, the intensity of the reflected radiation is decreased at wavelengths where the sample absorbs radiation, allowing a spectrum to be obtained that is similar to a transmission spectrum. Similarly, **internal reflectance cells** also are available for use with liquid samples, where the IR radiation penetrates a few micrometers into the liquid before being reflected back into an IR transmitting crystal in contact with the liquid. These types of cells are especially useful for samples such as aqueous liquids that absorb strongly in the mid-IR region.

Transmission spectra can be obtained from gas samples using a sealed 2–10-cm glass cell with IR transparent windows. For trace analysis, multiple-pass cells are available that reflect the IR beam back and forth through the cell many times to obtain pathlengths as long as several meters. FTIR instruments also can be interfaced to a gas chromatograph, to obtain spectra of compounds eluting from the chromatography column.

Commercial instruments are also available in which a **microscope** is interfaced to a FTIR spectrometer. The IR beam can be focused through the microscope onto a thin specimen mounted on a microscope slide. The IR spectrum then can be obtained from a very small area of the sample that measures only a few micrometers on each side. By moving the microscope stage, a profile of spectra across the sample can be obtained and used to evaluate the homogeneity of the sample. Mid-infrared imaging instruments are also available that use an array detector, similar to a digital camera, to obtain an image of the sample at different frequencies.

23.3.2 Applications of Mid-IR Spectroscopy

23.3.2.1 Absorption Bands of Organic Functional Groups

The wavelength bands where a number of important organic functional groups absorb radiation in the mid-IR region are shown in Table 23-1.

23.3.2.2 Presentation of Mid-IR Spectra

Spectra are normally presented with either wavenumbers or wavelengths plotted on the *x*-axis and either percent transmittance or absorbance plotted on the

23-1 table **Mid-IR Absorption Frequencies of Various Organic Functional Groups**

Group	*Absorbing Feature*	*Frequency* (cm^{-1})
Alkanes	−CH stretch and bend	3000–2800
	$-CH_2$ and $-CH_3$ bend	1470–1420 and 1380–1340
Alkenes	Olefinic −CH stretch	3100–3000
Alkynes	Acetylenic −CH stretch	3300
Aromatics	Aromatic −CH stretch	3100–3000
	−C=C− stretch	1600
Alcohols	−OH stretch	3600–3200
	−OH bend	1500–1300
	C−O stretch	1220–1000
Ethers	C−O asymmetric stretch	1220–1000
Amines	Primary and secondary −NH stretch	3500–3300
Aldehydes and ketones	−C=O stretch	1735–1700
	−CH (doublet)	2850–2700
Carboxylic acids	−C=O stretch	1740–1720
Amides	−C=O stretch	1670–1640
	−NH stretch	3500–3100
	−NH bend	1640–1550

y-axis. The mid-IR spectrum of polystyrene is shown in Fig. 23-3 and is typical of the common method of presentation of IR spectra.

23.3.2.3 Qualitative Applications

The center frequencies and relative intensities of the absorption bands can be used to identify specific functional groups present in an unknown substance. A substance also can be identified by comparing its mid-IR spectrum to a set of **standard spectra** and determining the closest match. Spectral libraries are available from several sources, but probably the largest collection of standards is the Sadtler Standard Spectra (Sadtler Division of Bio-Rad, Inc., Philadelphia, PA). Standard spectra are now commonly stored in digital format to allow searching by computer algorithm to determine the best match with an unknown compound. Common food applications include the identification of flavor and aroma compounds, particularly when FTIR measurements are coupled with gas chromatography. IR spectra also are useful for obtaining positive identification of packaging films.

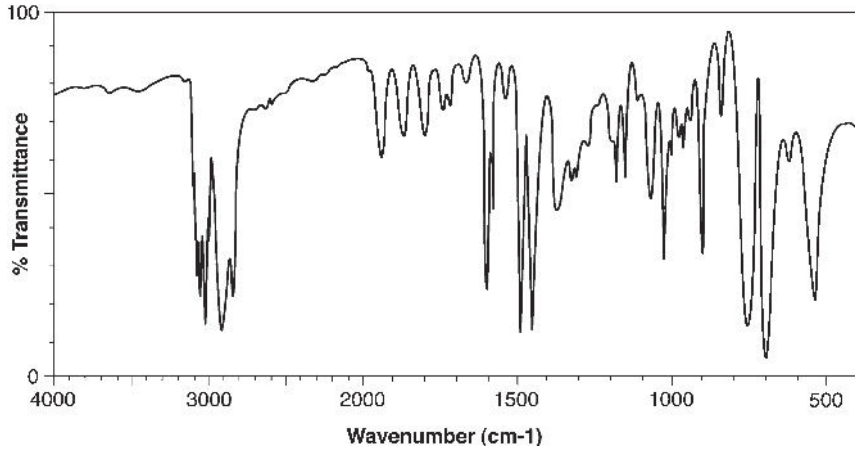
23.3.2.4 Quantitative Applications

IR spectroscopic measurements obey **Beer's law**, although deviations may be greater than in UV-Vis spectroscopy due to the low intensities of IR sources, the low sensitivities of IR detectors, and the relative narrowness of mid-IR absorption bands. However, quantitative measurements can be successfully made. Perhaps the most extensive use of this technique is in the **infrared milk analyzers**, which have the ability to analyze hundreds of samples per hour. The fat, protein, and lactose contents of milk can be determined simultaneously with one of these instruments. The ester carbonyl groups of lipid absorb at 5.73 µm $(1742\,cm^{-1})$, the amide groups of protein at 6.47 µm $(1348\,cm^{-1})$, and the hydroxyl groups of lactose at 9.61 µm $(1046\,cm^{-1})$. These automated instruments homogenize the milk fat globules to minimize light scattering by the sample, and then pump the milk into a flow-through cell through which the IR beam is passed. In some instruments, the monochromator uses simple optical interference filters that pass only a single wavelength of radiation through the sample, and the filter is selected depending on which constituent the operator wishes to measure. The instrument is calibrated using samples of known concentration to establish the slope and intercept of a Beer's law plot. Newer analyzers use an FTIR instrument to measure the absorbance at many wavelengths simultaneously, and then apply a **multiple linear regression** (MLR) or **partial least squares** (PLS) **regression** equation to predict the concentration of each constituent from the absorbance values at selected wavelengths. Regression techniques are described in more detail in Sect. 23.4. Official methods have been adopted for the IR milk analyzers, and specific procedures for operation of these instruments are given (1,2).

Commercial instruments also are available for measuring the fat content of emulsified meat samples by IR spectroscopy. A number of additional applications have been reported in recent years, particularly with respect to the characterization of fats and oils, including measurement of the degree of unsaturation, determination of *cis* and *trans* contents, and identification of the source of olive oils (3,4).

23.3.3 Raman Spectroscopy

Raman spectroscopy is a vibrational spectroscopic technique that is complementary to IR measurements (5). When a photon of light collides with a molecule, the collision can result in the photon being scattered. The collision can either be elastic, where kinetic energy is conserved, or inelastic, where energy is lost. When the photon interacts with the molecule, the energy of the molecule is raised to an unstable virtual state. When most molecules return to their ground state, they return to the lowest vibrational state, and the scattered photon has the same energy as the incident light. This is known as **Rayleigh scattering**. However,



23-3 figure

Mid-IR spectra of native and partially-hydrogenated soybean oils measured by ATR. Frequency in wavenumbers is plotted on the *x*-axis, with intensity on the *y*-axis. The bands just above 3000 cm^{-1} indicate the presence of unsaturated hydrocarbons in the molecules, while the strong −CH bands just below 3000 cm^{-1} indicate that saturated hydrocarbons are present in large amounts. The sharp band between 1700 and 1750 cm^{-1} arises from the carbonyls in the ester linkages between the fatty acids and glycerol backbone. Other −CH bands can be observed below 1500 cm^{-1}, including a −CH band exclusively associated with trans double bonds at 960 cm^{-1}.

a few molecules may return to a higher vibrational state. For those molecules, the scattered light will have less energy (lower frequency) than the incident light. The difference in frequency between the incident and scattered light is equal to the frequency of vibration of the molecule. This is known as **Raman scattering**. For Raman scattering to occur, a molecule must undergo a change in polarizability, but does not need to undergo a change in dipole moment. Thus, symmetrical vibrations that cannot be observed by IR spectroscopy can be observed by Raman. Raman is complementary to IR spectroscopy in that some vibrations are only Raman active, some are only IR active, and some are both.

In Raman spectroscopy, the intensity of the scattered light depends on the intensity of the source; therefore, a laser is most commonly used as the source of radiation. To minimize interference from fluorescence, near-infrared lasers are frequently used. The intensity of the scattered light is measured at different frequencies from the incident light, and a plot of $\Delta\nu$ in cm^{-1} vs. intensity is made to obtain the Raman spectrum.

Quantitative analysis can be performed with Raman spectroscopy, as the intensity of light scattered at a specific frequency is directly proportional to the concentration of scattering molecules present. Raman spectroscopy is applicable to both solid and liquid samples, often with minimal sample preparation for solids. Water is a very weak Raman scatterer, allowing low concentrations of organic molecules in aqueous solution to be measured. Conversely, water is a very strong IR absorber, which increases the complexity of applying IR techniques to aqueous samples. Finally, the Raman spectrum of a compound is often less complex than its mid or near-IR spectrum. These factors make Raman spectroscopy a useful alternative to IR spectroscopy. Applications of Raman spectroscopy to food analysis have been reviewed in the literature (6).

23.4 NEAR-INFRARED SPECTROSCOPY

Measurements in the **near-IR** (NIR) spectral region (0.7–2.5 µm, equal to 700–2500 nm) are more widely used for quantitative analysis of foods than are mid-IR measurements. Several commercial instruments are available for compositional analysis of foods using NIR spectroscopy. A major advantage of NIR spectroscopy is its ability to measure directly the composition of solid food products by use of diffuse reflection techniques.

23.4.1 Principles

23.4.1.1 Principles of Diffuse Reflection Measurements

When radiation strikes a solid or granular material, part of the radiation is reflected from the sample surface. This mirror-like reflection is called **specular reflection**, and gives little useful information about the sample. Most of the specularly reflected radiation is directed back toward the energy source. Another portion of the radiation will penetrate through the surface of the sample and be reflected off several sample particles before it exits the sample. This is referred to as **diffuse reflection**, and this diffusely reflected

radiation emerges from the surface at random angles through 180°. Each time the radiation interacts with a sample particle, the chemical constituents in the sample can absorb a portion of the radiation. Therefore, the diffusely reflected radiation contains information about the chemical composition of the sample, as indicated by the amount of energy absorbed at specific wavelengths.

The amount of radiation penetrating and leaving the sample surface is affected by the size and shape of the sample particles. Compensation for this effect may be achieved by grinding solid or granular materials with a sample preparation mill to a fine, uniform particle size, or by applying mathematical corrections when the instrument is calibrated (7).

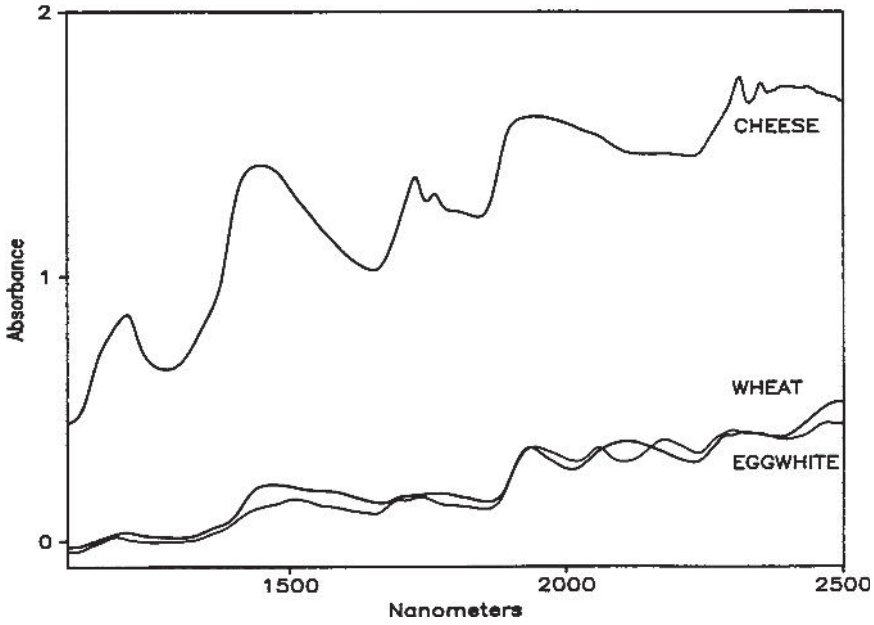
23.4.1.2 Absorption Bands in the NIR Region

The absorption bands observed in the NIR region are primarily overtones and combinations. Therefore, the absorptions tend to be weak in intensity. However, this is actually an advantage, since absorption bands that have sufficient intensity to be observed in the NIR region arise primarily from functional groups that have a hydrogen atom attached to a carbon, nitrogen, or oxygen, which are common groups in the major constituents of food such as water, proteins, lipids, and carbohydrates. Table 23-2 lists the absorption bands associated with a number of important food constituents.

The absorption bands in the NIR region tend to be broad and frequently overlap, yielding spectra that are quite complex. However, these broad bands are especially useful for quantitative analysis. Typical NIR spectra of wheat, dried egg white, and cheese are shown in Fig. 23-4. Note that strong absorption bands associated with the −OH groups of water are centered at ca. 1450 and 1940 nm. These bands are the dominant features in the spectrum of cheese, which contains 30–40% moisture, and they are still prominent even in the lower moisture wheat and egg white samples. Bands arising from the −NH groups in protein

23-2 table **Near-IR Absorption Bands of Various Food Constituents**

Constituent	*Absorber*	*Wavelength (nm)*
Water	−OH stretch/deformation combination	1920–1950
	−OH stretch	1400–1450
Protein – peptides	−NH deformation	2080–2220 and 1560–1670
Lipid	Methylene −CH stretch	2300–2350
	$-CH_2$ and $-CH_3$ stretch	1680–1760
Carbohydrate	C−O, O−H stretching combination	2060–2150



23-4 figure NIR spectra of cheese, wheat, and dried egg white plotted as $\log(1/R)$ vs. wavelength in nm.

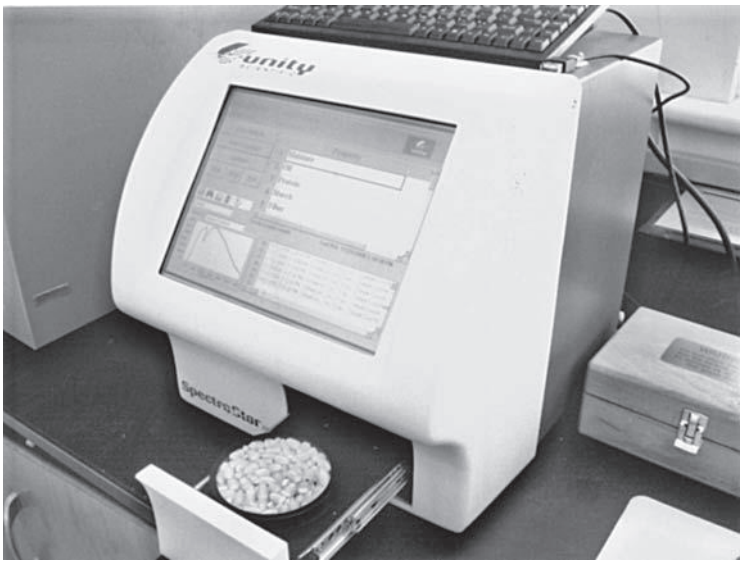
can be observed at 2060 nm and 2180 nm in the egg white spectrum but are partially obscured by a starch absorption band, centered at 2100 nm, in the wheat sample. Relatively sharp absorption bands arising from −CH groups in lipid can be observed at 2310 and 2350 nm, and another band from these groups is seen around 1730 nm. The 1730-nm band overlaps a weak protein absorption. The lipid bands are distinctly observable in the cheese spectrum.

23.4.2 Instrumentation

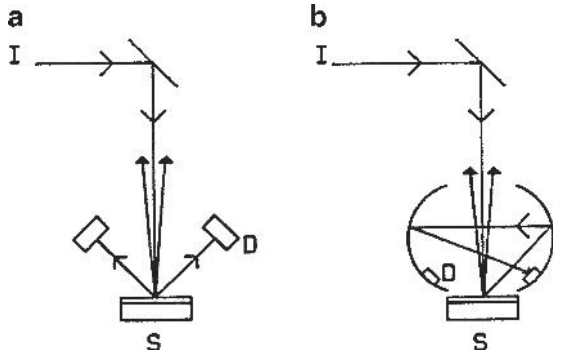
A commercial NIR spectrometer is shown in Fig. 23-5. The radiation source in most NIR instruments is a tungsten-halogen lamp with a quartz envelope, similar to a projector lamp. These lamps emit significant amounts of radiation in both the visible and NIR spectral regions. Semiconductor detectors are most commonly used in NIR instruments, with silicon detectors used in the 700–1100-nm range, and lead sulfide used in the 1100–2500-nm region. In situations for which a rapid response to changing light intensity is needed, such as in online monitoring, indium–gallium–arsenide (InGaAs) detectors can be used. Many InGaAs detectors are limited to a maximum wavelength of 1700 nm, although commercial InGaAs detectors with a range extended to longer wavelengths are now available. Most commercial NIR instruments use monochromators, rather than interferometers, although some commercial instruments are now using FT technology. Monochromator-based instruments may be of the scanning type, in which a grating is used to disperse the radiation by wavelength, and the grating is rotated to impinge a single wavelength (or more appropriately, a narrow band of wavelengths) onto a sample at any given time. Using this arrangement, it takes several seconds to collect a spectrum from a sample over the entire NIR region. Some rapid scanning instruments impinge light over the entire NIR region onto the sample, with the reflected or transmitted light then directed onto a fixed grating that disperses the light by wavelength, and also focuses it onto a multichannel array detector that measures all wavelengths at once. These instruments can obtain a spectrum from a sample in less than 1 s.

Instruments dedicated to specific applications can use optical interference filters to select 6–20 discrete wavelengths that can be impinged on the sample. The filters are selected to obtain wavelengths that are known to be absorbed by the sample constituents. The instrument inserts filters one at a time into the light beam to direct individual wavelengths of radiation onto the sample.

Either **reflection** or **transmission** measurements may be made in NIR spectroscopy, depending on the type of sample. In the reflection mode, used primarily for solid or granular samples, it is desirable to measure only the diffusely reflected radiation that contains information about the sample. In many instruments, this is accomplished by positioning the detectors at a 45° angle with respect to the incoming IR beam, so that the specularly reflected radiation is not measured (Fig. 23-6a). Other instruments use an integrating sphere, which is a gold-coated metallic sphere with the detectors mounted inside (Fig. 23-6b). The sphere collects the diffusely reflected radiation coming at various angles from the sample and focuses it onto the detectors. The specular component escapes from the



23-5 figure A modern commercial NIR instrument. (Photograph courtesy of the University of Nebraska-Lincoln.)



23-6 figure Typical instrument geometries for measuring diffuse reflectance from solid food samples. Radiation from the monochromator (I) is directed by a mirror onto the sample (S). Diffusely reflected radiation is measured directly by detectors (D) placed at a 45° angle to the incident beam (**a**) or is collected by an integrating sphere and focused onto the detectors (**b**). In both cases, the specularly reflected radiation is not measured.

sphere through the same port by which the incident beam enters and strikes the sample.

Samples are often prepared by packing the food tightly into a cell against a quartz window, thereby providing a smooth, uniform surface from which reflection can occur. Quartz does not absorb in the NIR region. At each wavelength, the intensity of light reflecting from the sample is compared to the intensity reflected from a nonabsorbing reference, such as a ceramic or fluorocarbon material. Reflectance (R) is calculated by the following formula:

$$R = I/I_0 \quad [4]$$

where:

I = intensity of radiation reflected from the sample at a given wavelength

I_0 = intensity of radiation reflected from the reference at the same wavelength

Reflectance data are expressed most commonly as $\log(1/R)$, an expression analogous to absorbance in transmission spectroscopy. Reflectance measurements also are expressed sometimes as differences, or derivatives, of the reflectance values obtained from adjacent wavelengths:

$$(\log R_{\lambda_2} - \log R_{\lambda_1}) \quad [5]$$

or

$$(2\log R_{\lambda_2} - \log R_{\lambda_1} - \log R_{\lambda_3}) \quad [6]$$

These derivative values are measures of the changes in slope of the spectrum, where λ_1, λ_2, and λ_3 are adjacent wavelengths typically separated by 5–20 nm, with the higher numbers representing longer wavelengths.

Transmission measurements also can be made in the NIR region, and this is usually the method of choice for liquid samples. A liquid is placed in a quartz cuvette and the absorbance measured at the wavelengths of interest. Transmission measurements also can be taken from solid samples, but generally only in the 700–1100-nm range. In this wavelength region, the absorption bands are higher overtones that are very weak, allowing the radiation to penetrate through several millimeters of a solid sample. The use of transmission measurements can minimize the degree of sample preparation needed. Since the IR beam passes through the entire sample, the need for a smooth, homogeneous sample surface is reduced.

NIR energy can be transmitted through a fiber optic cable some distance from the monochromator or interferometer, allowing reflection or transmission measurements to be made remotely from the instrument. This is very useful if we wish to take measurements in a processing plant environment. Commercial probes are available that can be inserted directly into bulk granular materials, or inserted into a pipe carrying a liquid.

As with mid-IR, NIR imaging instruments are also now commercially available. These instruments use an array detector so that a digital image of a food sample can be obtained at various wavelengths, or a spectrum can be obtained from a single pixel in a digital image. This technique is often referred to as hyperspectral imaging, and holds much potential for evaluating sample heterogeneity, or identifying small features or contaminants on an intact food sample.

23.4.3 Quantitative Methods Using NIR Spectroscopy

NIR instruments can be calibrated to measure various constituents in food and agricultural commodities. Because of the overlapping nature of the NIR absorption bands, it is usually necessary to take measurements at two or more wavelengths to quantitate a food component reliably. Multivariate statistical techniques are used to relate the spectral data collected at multiple wavelengths to the concentration of the component of interest in the food. The simplest statistical technique used is MLR, which applies an equation of the following form to predict the amount of a constituent present in the food from the spectral measurements:

$$\%\,\text{constituent} = z + a\log(1/R_{\lambda 1)} + b\log(1/R_{\lambda 2}) + c\log(1/R_{\lambda 3}) + \ldots \quad [7]$$

where each term represents the spectral measurement at a different wavelength multiplied by a corresponding coefficient. Each coefficient and the intercept (z)

are determined by the multivariate regression analysis. Absorbance or derivatized reflectance data also can be used in lieu of the $\log(1/R)$ format. Use of derivatized reflectance data has been found to provide improved results in some instances, particularly with samples that may not have uniform particle sizes. Other mathematical techniques also are available that can be applied to the reflectance data to correct for the effects of nonuniform particle size (7).

23.4.3.1 Calibration Methods Using Multiple Linear Regression

The first step in calibrating an NIR instrument is to select a set of calibration, or training, samples. The samples should be representative of the products that will be analyzed, contain the constituent of interest at levels covering the range that is expected to be encountered, and have a relatively uniform distribution of concentrations across that range. The calibration samples are analyzed by the classical analytical method normally used for that constituent, and spectral data also are obtained on each sample with the NIR instrument at all available wavelengths. All data are stored into computer memory. MLR is then used to select the optimum wavelengths for measurement and the associated coefficients for each wavelength. Wavelengths are selected based on statistical significance by using a step forward or reverse stepwise regression procedure or by using a computer algorithm that tests regressions using all possible combinations of two, three, or four wavelengths to determine the combination that provides the best results. Most calibrations will use between two and six wavelengths, and one should always check to make certain that the wavelengths chosen on the basis of statistical significance also make sense from a spectroscopic standpoint. Calibration results are evaluated by comparing the multiple correlation coefficients, *F*s of regression, and standard errors for the various equations developed. It is desirable to maximize the correlation coefficient (generally R should be >0.9) and minimize the standard error. A calibration always should be tested by using the instrument to predict the composition of a set of test samples that are completely independent of the calibration set and comparing the results obtained to the classical method.

23.4.3.2 Calibration Development Using Full Spectrum Methods

Calibration techniques such as **PLS regression** and **principal components regression** (PCR) have been developed that use information from all wavelengths in the entire NIR spectrum, rather than a few selected wavelengths, to predict sample composition. PLS and PCR use data reduction techniques to extract from a large number of variables (i.e., reflectance or absorbance measurements at many wavelengths) a much smaller number of new variables that account for most of the variability in the samples. These new variables then can be used to develop a regression equation to predict the amount of a constituent in samples of a food. In PLS and PCR methods, it is not necessary to eliminate spectral information, as it is in MLR where only a limited number of wavelengths are used. PLS and PCR methods are reported to yield improved results for some samples (8).

Artificial neural networks have also recently been used to predict composition from NIR spectra. Neural networks may have some advantages over the linear regression techniques for dealing with highly complex samples, or samples from diverse geographic regions.

23.4.4 Qualitative Analysis by NIR Spectroscopy

NIR spectroscopy also can be used to classify a sample into one of two or more groups, rather than to provide quantitative measurements. **Discriminant analysis** techniques can be used to compare the NIR spectrum of an unknown sample to the spectra of samples from different groups. The unknown sample then is classified into the group to which its spectrum is most similar. While this technique has been more widely used in the chemical and pharmaceutical industries for raw material identification, it has also been used for food applications, including the classification of wheat as hard red spring or hard red winter (9), the identification of orange juice samples from different sources (10), authentication of the source of olive oils (11), and discrimination of beef by breed and muscle type (12).

23.4.5 Applications of NIR Spectroscopy to Food Analysis

Theory and applications of NIR spectroscopy to food analysis have been discussed in several publications (13–16). The technique is widely used throughout the grain, cereal products, and oilseed processing industries. NIR techniques using measurements from ground or whole grain samples have been adopted as approved methods of analysis by AACC International (17) for measuring protein in barley, oats, rye, triticale, wheat, and wheat flour, as well as moisture, protein, and oil in soybeans. These approved methods describe the instruments available for making these measurements, including a list of current manufacturers with contact information in Method 39-30, as well as the proper techniques for preparing samples and calibrating the instruments. NIR instruments now are used by

the official grain inspection agencies in both the US and Canada for measuring protein, moisture, and oil in cereals and oilseeds.

Components, such as protein and dietary fiber, can be determined successfully in a number of cereal-based foods using NIR spectroscopy (18–20). Modern instruments and calibration techniques allow a wide variety of products, such as cookies, granola bars, and ready-to-eat breakfast foods, to be analyzed using the same calibration.

NIR spectroscopy also can be used for numerous other commodities and food products. The technique has been used successfully to evaluate composition and quality of red meats and processed meat products (21–23), poultry (24), and fish (25). NIR spectroscopy is useful also for analyzing a number of dairy products and nondairy spreads, including measuring moisture in butter and margarine (26); moisture, fat, and protein in cheese (27, 28); and lactose, protein, and moisture in milk and whey powders (29). NIR techniques also have shown promise for measuring total sugars and soluble solids in fruits, vegetables, and juices (30–32), are being used commercially for monitoring the sugar content in corn sweeteners (33), and can be used to quantitate sucrose and lactose in chocolate (34).

NIR spectroscopy also is showing potential for measuring specific chemical constituents in a food that affect its end-use quality, for monitoring changes that occur during processing or storage, and for directly predicting processing characteristics of a commodity that are related to its chemical composition. Examples include determining the amylose content in rice starch, an important determinant of rice quality (35,36), monitoring peroxide value in vegetable oils (37), monitoring degradation of frying oils (38), and predicting corn processing quality (39,40).

These are only a few examples of current applications. If a substance absorbs in the NIR region, and is present at a level of a few tenths of a percent or greater, it has potential for being measured by this technique. The primary advantage of NIR spectroscopy is that once the instrument has been calibrated, several constituents in a sample can be measured rapidly (from 30 s to 2 min) and simultaneously. To measure multiple constituents, a calibration equation for each constituent is stored into the memory of the instrument. Measurements are taken at all wavelengths needed by the calibrations, and each equation then is solved to predict the constituents of interest. No sample weighing is required, and no hazardous reagents are used or chemical waste generated. It is also adaptable for **online measurement systems** (41). Disadvantages include the high initial cost of the instrumentation, which may require a large sample load to justify the expenditure, and the fact that specific calibrations may need to be developed for each product measured. Also, the results produced by the instrument can be no better than the data used to calibrate it, which makes careful analysis of the calibration samples of highest importance.

23.5 SUMMARY

IR spectroscopy measures the absorption of radiation in the near- ($\lambda = 0.8$–$2.5\,\mu m$) or mid- ($\lambda = 2.5$–$15\,\mu m$) IR regions by molecules in food or other substances. IR radiation is absorbed as molecules change their vibrational energy levels. Mid-IR spectroscopy is especially useful for qualitative analysis, such as identifying specific functional groups present in a substance. Different functional groups absorb different frequencies of radiation, allowing the groups to be identified from the spectrum of a sample. Quantitative analysis also can be achieved by mid-IR spectroscopy, with milk analysis being a major application. NIR spectroscopy is used most extensively for quantitative applications, using either transmission or diffuse reflection measurements that can be taken directly from solid foods. By using multivariate statistical techniques, NIR instruments can be calibrated to measure the amounts of various constituents in a food sample based on the amount of IR radiation absorbed at specific wavelengths. NIR spectroscopy requires much less time to perform quantitative analysis than do many conventional wet chemical or chromatographic techniques.

23.6 STUDY QUESTIONS

1. Describe the factors that affect the frequency of vibration of a molecular functional group and thus the frequencies of radiation that it absorbs. Also, explain how the fundamental absorption and overtone absorptions of a molecule are related.
2. Describe the essential components of an FT mid-IR spectrometer and their function, and compare the operation of the FT instrument to a dispersive instrument. What advantages do FT instruments have over dispersive IR spectrophotometers?
3. Describe the similarities and differences between mid-infrared spectroscopy and Raman spectroscopy.
4. Of the three antioxidants butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate, which would you expect to have a strong IR absorption band in the 1700–1750 cm^{-1} spectral region? Look up these compounds in a reference book if you are uncertain of their structure.
5. Describe the two ways in which radiation is reflected from a solid or granular material. Which type of reflected radiation is useful for making quantitative measurements on solid samples by NIR spectroscopy? How are NIR

reflectance instruments designed to select for the desired component of reflected radiation?

6. Describe the steps involved in calibrating an NIR reflectance instrument to measure the protein content of wheat flour. Why is it usually necessary to make measurements at more than one wavelength?

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