Near-infrared Spectroscopy in Food Analysis

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Near-infrared (NIR) spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range 780–2500 nm. NIR spectra of foods comprise broad bands arising from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C−H, O−H and N−H chemical bonds. The concentrations of constituents such as water, protein, fat and carbohydrate can in principle be determined using classical absorption spectroscopy. However, for most food samples, this chemical information is obscured by changes in the spectra caused by physical properties such as the particle size of powders. This means that NIR spectroscopy becomes a secondary method requiring calibration against a reference method for the constituent of interest. As a consequence of the physics of diffuse transmittance and reflectance and the complexity of the spectra, calibration is normally carried out using multivariate mathematics (chemometrics).

NIR spectroscopy is used routinely for the compositional, functional and sensory analysis of food ingredients, process intermediates and final products.

The major advantage of NIR is that usually no sample preparation is necessary, hence the analysis is very simple and very fast (between 15 and 90 s) and can be carried out on-line. One of the strengths of NIR technology is that it allows several constituents to be measured concurrently. In addition, for each fundamental vibration there exists a corresponding series of overtone and combination bands with each successive overtone band approximately an order of magnitude less intense than the preceding one. This provides a built-in dilution series which allows several choices of absorptions of different intensity containing the same chemical information. Finally, the relatively weak absorption due to water enables high-moisture foods to be analyzed.

The major limitation of NIR spectroscopy in food analysis is its dependence on less-precise reference methods.

1 INTRODUCTION

The NIR forms that part of the electromagnetic spectrum in the wavelength range 780 nm to 2500 nm (Figure 1). NIR, like all radiation, behaves as a wave with the properties of *simple harmonic motion* which may be defined in terms of two properties:

- the frequency of vibration
$$= \text{no. of times the wave pattern is repeated in 1 s}$$
$$= \text{angular velocity}/2$$

- the wavelength $= \dfrac{\text{velocity of light}}{\text{frequency}}$

Chemical bonds between atoms in molecules vibrate and to a first approximation this vibration behaves as a simple harmonic motion. The motion of each atom may be treated as an independent vibration with respect to a fixed centre of mass of the molecule rather like a mass attached to a spring. The vibration frequency is a function of the masses of the two atoms m_1 and m_2 and the strength of the bond k and there is a parabolic relationship between potential energy and interatomic distance.
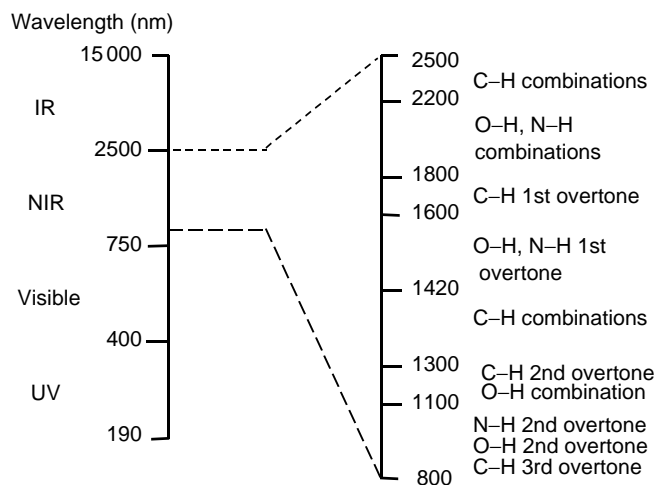
Wavelength (nm)

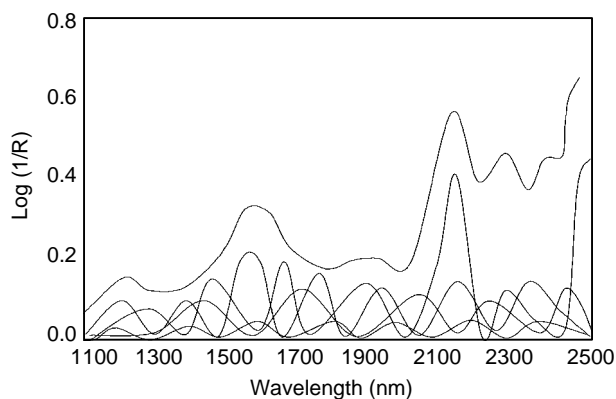**Figure 1** Principal types of NIR absorption bands and their locations.

Figure 2 A typical spectrum of a powdered cereal sample (upper curve) and some of its constituent absorptions.

When the frequency of the radiation matches that of the vibrating molecule, there will be a net *transfer of energy* from the radiation to the molecule which can be measured as a plot of energy versus wavelength called a spectrum (Figure 2). However, energy is not transferred continuously but in discrete packets called quanta. In consequence, molecules can only occupy discrete energy levels defined by whole numbers $0, 1, 2, \ldots$ and the energy change required for a transition between two levels is directly related to the frequency of the energy by multiplying by h (Planck's constant). For simple harmonic motion, the absorption or emission of energy can only take place if it matches a transition between any pair of adjacent energy levels. Since almost all the molecules in a sample will normally occupy the lowest energy level 0, this means that the only possible transition is from 0 to 1, called the fundamental.

The simple harmonic model does not adequately describe the vibrational motion of chemical bonds

because at one extreme atoms repel one another the closer they approach and at the other extreme the molecule dissociates. For this more realistic model, transitions from energy level 0 to 2, 0 to 3 etc. become possible and these are referred to as first, second etc. *overtones* with frequencies corresponding approximately to twice, three times etc. that of the fundamental. The frequencies of many overtone bands are in the NIR region. The greater the deviation of a particular bond system from harmonicity, the greater the intensity of overtone bands and the most anharmonic bonds are those involving the lightest atom, hydrogen, and a heavier atom such as carbon, nitrogen or oxygen.

While each frequency of vibration of a complex molecule generally involves oscillation of all the atoms, those atoms undergoing large amplitude motions are often limited to a particular functional group, e.g. $-OH$, $-NH_2$ etc. The characteristic frequency is similar for the same functional group where it appears in different molecules. This concept of *characteristic group frequencies* is the basis of analysis by infrared (IR) and, because each fundamental will be associated with its own potential energy curve and its own set of overtones, of analysis by NIR also.

The number of bands a given molecule will produce can be calculated from the number of coordinates required to describe the total momentum of the molecule. $3n$ coordinates describe the location in space of a molecule of n atoms of which 3 describe the translational and 3 the vibrational motion (2 if linear) of the molecule. There are therefore $3n - 6$ ($3n - 5$ if linear) possible *modes of vibration* but some of these may be the same. Furthermore, absorption can only occur if the molecular vibration is accompanied by a change in dipole moment such as with the hydrogen chloride molecule.

Another type of absorption band occurs in the NIR region. *Combination bands* arise by interaction of two or more vibrations taking place simultaneously and the frequency of a combination band is simply the sum of multiples of the relevant fundamental frequencies. Thus, a triatomic molecule with 3 fundamental bands could have 3 first overtones, 3 second overtones etc. and 6 combination bands. It soon becomes obvious that NIR spectra are much more complex than IR spectra. Nevertheless, overtones and combinations can still be assigned to group frequencies.

2 INSTRUMENTATION

Successful application of NIR depends on the correct choice of instrument. There are three different types available (described in detail by Osborne et al.[1])

2.1 Monochromators

Grating monochromators are used to measure the full visible and NIR spectrum and may be used in transmittance or reflectance mode. They are therefore the most versatile instruments. Monochromators are generally used for research or when a wide range of different applications is required. Three different detectors are available: a silicon detector covers the range 400–1100 nm, an indium gallium arsenide covers the range 800–1700 nm and a lead sulfide the range 1100–2500 nm. Some instruments contain both silicon and lead sulfide detectors (giving them a wavelength range 400–2500 nm), some only the lead sulfide. The latter would be restricted to measurement of powdered or granular samples by diffuse reflectance (see section 3.2) or transmittance via fiber-optic probes. At least one model of NIR monochromator has been designed for on-line use via either fiber-optics or a powder sampler.

Another type of dispersive monochromator used in NIR instruments is the acousto-optically tunable filter (AOTF). An AOTF comprises a crystal of TeO_2 through which a plane travelling acoustic wave is generated at right angles to the incident light beam. This causes the crystal to behave as a longitudinal diffraction grating with a periodicity equal to the wavelength of sound across the material. The main advantages of AOTF over grating instruments is their mechanical simplicity (i.e. no moving parts) and their wavelength stability.

2.2 Diode Array Spectrometers

Diode array spectrometers employ an array of IR-emitting diodes. These function as both the light source and the wavelength selection system. Diode array instruments typically cover the range 400–1700 nm. They have the advantages that the measurement is very fast (e.g. one spectrum per second) and noninvasive. These features are particularly useful where a high sample throughput or ultra-rapid on-line measurements are required.

2.3 Filter Instruments

The simplest and cheapest NIR instruments are based on a limited number, usually between six and twenty, of interference filters. These filters are chosen to represent the absorptions used for the most popular applications, e.g. protein, moisture and oil in agricultural samples. Filter instruments are designed for a limited range of routine analyses, either in the laboratory or on-line.

3 SAMPLE PRESENTATION

The wide range of applications of NIR in food analysis is possible because of the different sample presentation techniques. There is a technique available for any type of liquid, slurry, powdered or solid sample.

3.1 Diffuse Transmittance

Radiation interacting with a sample may be absorbed, transmitted or reflected. In the classical spectroscopy experiment, reflection is eliminated so that the proportion of radiation attenuated by the sample may be measured as transmittance. *Beer's law* then defines a proportionality between transmittance and the product of concentration of the absorbing species and path length. For a clear transparent liquid sample such as beer, hot starch melts, wine or vegetable oil, the path length may be fixed by means of a static or flow-through sample cuvette or a pair of fiber-optic probes and a calibration developed using samples of known concentrations. For example, Halsey[2] used standard solutions of ethanol in water to develop a calibration for alcohol content of beer. It should be noted that, owing to the relatively weak intensities of NIR absorption bands, samples such as vegetable oils may be analyzed without dilution in a solvent.

Beer's law is only valid in the absence of light scatter in the sample. Scattering changes the path length through which the radiation passes and, because the amount of scattering varies from sample to sample, the path length cannot be defined. This type of experiment is known as *diffuse transmittance* (Figure 3), the most well-known example of which is liquid whole milk. The fat globules in the milk scatter light in the manner shown and invalidate Beer's law. Diffuse transmittance measurements are usually carried out in the 800–1100 nm region of the spectrum where the weak absorptions enable useful data to be obtained using thicknesses of 1–2 cm of samples such as meat, cheese or whole grain. Near-infrared transmittance (NIT) instruments are particularly applicable to the analysis of whole grains and a typical apparatus is shown schematically in Figure 4. A sample of grain is placed in a hopper from where aliquots are dispensed into the measurement chamber. When analysis is complete, the sample is discharged into a collection tray. This arrangement lends itself well to adaptation for on-line measurements (see section 5.1).
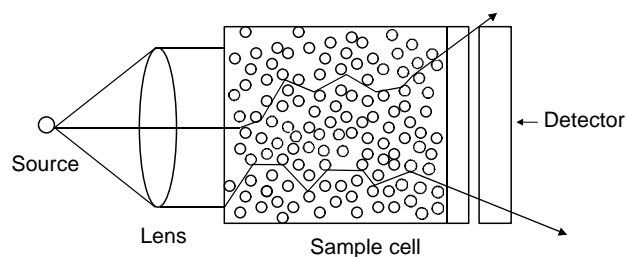

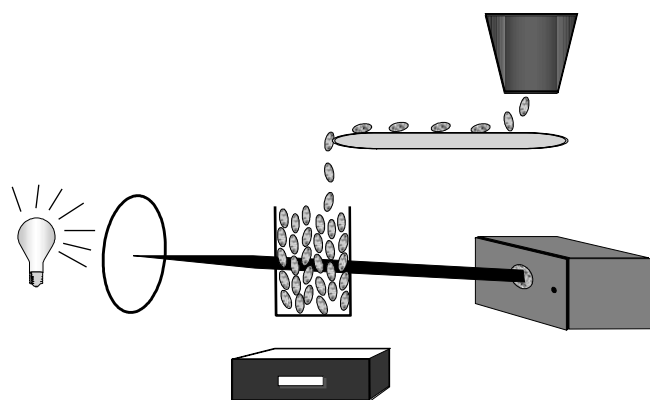**Figure 3** Diffuse transmittance spectroscopy.

Figure 4 Diffuse transmittance spectroscopy of a whole grain sample.

3.2 Diffuse Reflectance

For a smooth surface such as glass, most of the radiation is reflected from the surface by regular or *specular reflection* and no absorption takes place. In the 1100–2500 nm region, the amount of scattering makes the path length so great that transmittance through 1 cm of most samples is negligible. This situation is called *diffuse reflectance* because most of the incident radiation is reflected. If a matt surface reflects diffusely without penetration into the sample, like specular reflectance no absorption takes place. If, however, some of the radiation penetrates the surface when it reaches each particle it can be reflected, absorbed or transmitted. The net result is that the diffusely reflected radiation (R) can be empirically related to concentration (c) in an analogous way to Beer's law i.e. $\log 1/R = kc$ where k is a factor which incorporates both absorbtivity and path length.

A typical NIR diffuse reflectance experiment is shown in Figure 5. A powdered sample such as wheat meal is
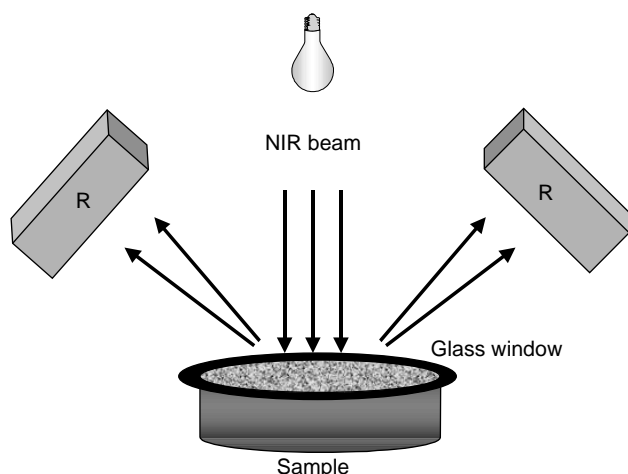

Figure 5 Diffuse reflectance analysis of a powdered sample.

packed into a 1 cm-deep sample cell and covered with a quartz window. The sample cell is then placed into the instrument where it is illuminated with NIR radiation and the reflected radiation measured by either a set of detectors set at 45° to the incident beam or a single detector onto which the radiation is focused by means of an integrating sphere. This configuration has been adapted for on-line measurement using a powder sampler for flour or milk powders.

The diffuse reflectance experiment can be adapted for use with liquids by placing a ceramic tile beneath the sample. In this sample presentation mode, known as *transflectance*, the radiation is transmitted through the sample, reflected from the ceramic then transmitted back through the sample before finally reaching the detector. Transflectance is thus a hybrid of transmittance and reflectance. *Interactance*, another hybrid of transmittance and reflectance, involves illumination and detection at laterally separated points on the sample's surface. It is normally accomplished using a fiber-optic probe in which one set of fiber-optic bundles carries the incident radiation and another carries the reflected radiation. This type of arrangement is particularly useful for large samples such as intact fruit.

3.3 On-line Samplers

Ideally, a sensor needs to be sufficiently compact and robust to be placed in the required position on-line. It needs to be relatively low cost, rapid and capable of measuring the desired analyte or property on an intact sample. Finally, it must be capable of accurate and stable calibration. NIR instruments meet these requirements. Since the applications are limited and predefined, filter instruments which fulfil the criteria of compactness, robustness and cost can usually be employed. NIR is inherently a rapid technique (as fast as one spectrum per second with diode array technology) and data from many measurements can be combined into a moving average so as to identify trends.

There are three types of NIR on-line analyzer.

3.3.1 Remote (Noncontact) Sensor

The first dedicated on-line NIR sensor was invented by Edgar and Hindle of Infrared Engineering Co in the UK. This is based on a sensing head, which is referred to as a "gauge", remote from the sample. This design has the advantages of a low cost of instrument and simplicity of installation but imposes severe constraints on instrument design as it is susceptible to interference from ambient light variations, dust build-up on the optical surfaces and atmospheric humidity variation. The Infrared Engineering gauge was therefore designed specifically for on-line application and constructed to

minimize such potential interferences. The Infrared Engineering MM55 Gauge is based on a non-contacting sensing head fixed approximately 200 mm from the product flow by mounting to the side of a section of spouting inclined at an angle of 60° from the horizontal. The gauge focuses light from a quartz halogen lamp into a parallel beam which is projected onto the product via a filter wheel which transmits narrow bands of energy at the desired NIR wavelengths. The reflected energy, which has been modulated by interaction with the product, is captured by a detector in the gauge. In this way, measurements on the moving flour stream are taken through a toughened glass window at a rate of up to five per second. Signals from the MM55 gauge are conveyed to an electronic processing unit which contains the calibration equation and produces the analytical result. This is passed to a strip recorder to provide a permanent record. Applications of the MM55 in the food industry include moisture determination on both continuous samples such as powders and discontinuous samples such as whole biscuits, control of protein in flour, monitoring of fat content of potato crisps and sorting of fruit.

Recently, in Australia, a novel application of NIR spectroscopy to the monitoring of bread dough development has been invented by Wesley et al.[3] The invention makes use of the high scan speed (one spectrum per second) of the Perten DA-7000 diode array instrument. In this case, the instrument was inverted over an open mixing bowl and the signal from the dough was recorded continuously without stopping the mixer. In this way, using second derivative data at defined wavelengths assigned to protein and water, an NIR mixing curve was obtained. The NIR mixing curves were in good agreement with curves obtained using measurement of mixer torque over a range of flour types and mixer conditions.

3.3.2 By-pass Sampler

To control the composition of the grist (wheat mixture) during the blending process, it is necessary to perform measurements on the grain itself because the time delay between blending the wheats and producing the final flour is too long for an effective feedback control loop. Whole grain NIR instruments generally employ a hopper and flow-through cell and are thus relatively easy to adapt to on-line use. An on-line version of the Infratec 1225 Whole Grain Analyzer is commercially available.

One of the first near-infrared on-line powder samplers (NIROS) was based on an InfraAlyzer 300B instrument which was split into two parts comprising the electronics unit which would be removed from the immediate vicinity of sampling and the optical unit which was to be modified for on-line sample presentation. This method of sample presentation was selected on the principle that the sample needed to be held stationary against the optics window during the measurement cycle and that the flour surface should be smooth, continuous and firmed under pressure. These conditions were met by taking direct samples from the flour stream, either from gravity-fed spouting or a positive pressure blowline, into a mechanism designed to reproduce the action of a human operator in packing flour into a sample cup.

NIR on-line samplers were first developed to measure the protein content of flour. This is still the most popular application and provides an excellent example of an NIR feedback control system. Dried gluten is commonly used, particularly in Europe, to replace wholly or partly the protein in flour which would otherwise be derived from high-protein wheat in the grist. The success of on-line NIR for monitoring flour protein content therefore led to its incorporation into a closed-loop control system for gluten addition to flour.[4] A mixing screw is installed between the gluten feed and the NIR sampler station from which a feedback signal controls the gluten feeder. The system has proved to be an efficient and accurate method of control of gluten addition to achieve a target protein content in the flour.

3.3.3 Fiber-optic Probe

Fiber-optic probes have the widest range of applications in on-line food analysis. Uses include dairy products, meat, fruit, beer and extruded snack foods.

Extrusion cooking is used to manufacture a wide range of products from cereals, including breakfast cereals, snack foods and pet foods. In these products, texture and density are key quality requirements which are controlled by the processing conditions summarized by the term "degree of cook". Conventionally, control is attempted by measurement of inputs such as the moisture content, screw speed, barrel temperature, etc. on the basis that the relationship between these and the product quality is understood. However, a more direct means of control is highly desirable. In contrast to breadmaking, where the product structure is largely controlled by protein development, in extrusion cooking it is the starch component of the cereal-based raw material which is the chief functional ingredient. Therefore, the aim was to use NIR to monitor changes in starch structure. Initially, products were prepared over a wide range of processing conditions and the extrudates freeze-dried and ground to powders prior to NIR spectroscopy. Since successful calibrations based on spectral features related to starch structure were obtained using the powdered samples,[5] the measurement was attempted on-line. A fiber-optic reflectance probe was installed in the die of the extruder and the light was transmitted through the melt and reflected back from a polished steel pillar;

in effect, therefore, a transflectance measurement was used. The spectral characteristics of the different forms of starch found in the hot melts close to the die of the extruder were identical to those of the powdered extrudates. This research has opened the way to an on-line process monitoring and control system for extrusion cooking.

4 CALIBRATION DEVELOPMENT

In NIR analysis, the calibration provides all three basic functions of analytical chemistry i.e. separation, identification and quantification. Therefore, derivation of the calibration equation for each application is the most crucial step in reliable and accurate analysis. There is, however, no unique method for deriving satisfactory calibration equations. The following sections provide an overview of the main techniques used and more detail can be found in Osborne et al.[1]

4.1 Data Preprocessing

Quantitatively the most important factor in the NIR reflectance spectrum is the particle size of the sample. $\log(1/R)$ increases with increasing particle size because the apparent path length becomes longer. Therefore, for example, the same wheat sample ground to different particle sizes will result in substantially different spectra. However, the effect is not additive but multiplicative, i.e. proportional to $\log(1/R)$. Since in reflectance spectra of foods and agricultural materials $\log(1/R)$ coincidentally increases with increasing wavelength, the effect of particle size appears to be a function of wavelength.[6,7]

Correction for particle size is accomplished empirically by multiple regression mathematics but many spectroscopists prefer to use derivatives. The first derivative

$$\frac{d(\log 1/R)}{d\lambda}$$

is the slope of the spectrum at wavelength λ and is calculated as the difference between $\log 1/R$ at two adjacent wavelengths or, more usually, two segments in which the data are smoothed (averaged) over a small region of the spectrum defined by the segment size. The difference between these wavelengths or segments is called the gap. The second derivative

$$\frac{d^2(\log 1/R)}{d\lambda^2}$$

is the difference between two adjacent first derivatives. Derivative mathematics is described using the notation (derivative order, gap, segment, second smoothing) e.g.

(2, 8, 8, 1) means a second derivative with a 16 nm gap and 16 nm segment.

Derivatives have two useful properties:

1. resolution of overlapping bands;
2. deconvolution of background (derivative ratios[6] correct for multiplicative effects).

The effect of particle size can also be overcome by multiplicative scatter correction[8] or standard normal variate and detrend.[9] The multiplicative scatter correction algorithm proceeds by computing the mean spectrum of the sample set and rotating each sample spectrum so that a regression line drawn through it aligns with a regression line drawn through the mean spectrum.

4.2 Sample Population Structuring

The NIR analysis of food and agricultural samples is based on calibration against a reference method using a set of samples which represent all the variability in the population from which they are drawn. The problem becomes one of identifying these samples. One set of criteria would be to obtain analytical data on a large database of samples and select those with the most variation in composition. However, this is an expensive option. The concept of population structuring was introduced by Shenk and Westerhaus.[10] The idea is to assemble a library containing the NIR spectra of a large number of samples and use discriminant analysis techniques to define the boundaries of the library. The spectra of further samples can then be examined to ascertain whether they belong to the population represented by the library and whether their spectra are different to those of existing samples. The spectral library can be used in several ways:

- to define the optimum calibration set;
- to verify that test samples match the calibration;
- to produce locally weighted calibrations for optimum measurement accuracy;
- to identify new samples with which to extend the calibration.

4.3 Regression Techniques

The usual method of calibration of NIR instruments is by multiple linear regression of the reference data on the spectral data. The difficulty in this is twofold. Firstly, NIR spectra contain a large amount of data whereas only a few terms should be included in a regression equation. Secondly, because of the effect of scatter, the raw NIR data are highly intercorrelated and this precludes the use of forward stepwise regression, the most common method of selection of terms in

multiple linear regression. For fixed filter instruments, the wavelengths for each application may be known. If not, there are only a few from which to select and is feasible to employ an all possible combinations strategy. For monochromator or diode array instruments on the other hand, more sophisticated methods are necessary. The use of mathematical pretreatments such as derivatives or multiplicative scatter correction (see section 4.1) would make forward stepwise selection more reliable but it is important to ensure that the selected wavelengths are consistent with known chemical absorptions. This can be done by comparing a plot of correlation versus wavelengths (Figure 6) with the spectrum of the constituent of interest.[11] However, this is not always possible. For example, functional properties do not usually have a defined spectral pattern. An increasingly popular approach, therefore, is to reduce the amount of data by principal components analysis then perform linear regression on the principal components. If the principal components are selected with regard to minimizing the correlation coefficient, the method becomes partial least squares (PLS). The end result is a calibration equation from which the constituent of interest may be calculated from a (usually) linear combination of spectral data. The equation has associated statistics which define the closeness of fit of the actual and predicted values to the least squares line. It is always important to plot a scatter graph as illustrated in Figure 7 in order to detect any aberrant data. Ideally, the scatter plot should contain data points distributed evenly about the line but within the confidence limits as shown in the example.

4.4 Qualitative Analysis

In applications such as authenticity testing (see section 5.8), where the aim is to classify rather than perform a quantitative determination, a qualitative or discriminant
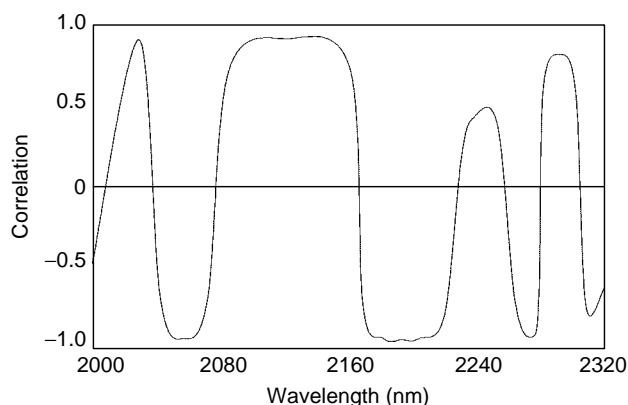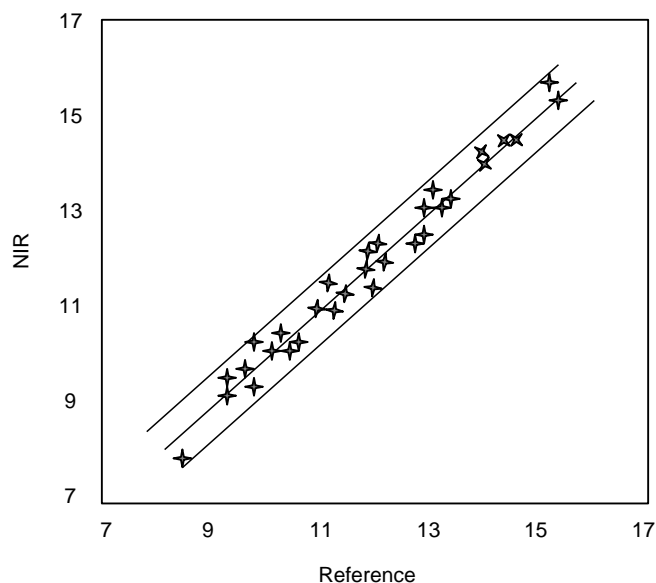
Figure 7 A typical scatter plot of NIR versus reference data.

analysis approach is often used. The mathematical approach to this problem is to develop a model which defines the mean and standard deviation of each sample type in multidimensional space then test to which group an unknown sample belongs. This test is based on a non-Euclidean distance measurement such as the Mahalanobis distance. The Mahalanobis distance is defined according to elliptical contours spreading out from the group centres. The mathematics and applications of discriminant analysis are described by Mark.[12] More sophisticated classification models have been achieved using artificial neural networks (ANNs). ANNs consist of input nodes which receive one piece of spectral data each and distribute it to a hidden layer of nodes where the data are transformed before being distributed again to a set of output nodes. The nodes simply provide the mechanism for distribution of the data through the hidden layer which may be considered a nonlinear function approximation machine. The crucial feature of ANNs is that they are adaptive. One example of the application of ANNs to a food classification problem has been published by Hervas et al.[13]

5 FOOD APPLICATIONS

5.1 Cereals and Cereal Products

NIR has been used for the quality testing of crossbred material from wheat breeding programs since the late 1970s. Because NIR is rapid, low cost per test and requires a relatively small quantity of sample, it is routinely used throughout the world to determine the protein content

Figure 6 Plot of correlation versus wavelength for a set of calibration samples.

of wheat to allow breeders to screen large numbers of lines for this key characteristic. In addition, NIR is widely used to replace some of the chemical tests necessary as part of a quality testing program (Table 1). In particular, NIR hardness and moisture measurements may be used to determine the conditioning requirements prior to test milling while protein and moisture may be determined on the resulting flour.

The ultimate application of NIR analysis in a wheat breeding program would be direct prediction of functional quality including flour yield, damaged starch, water absorption, dough development time, extensibility and loaf volume. Although some breeding programs are using NIR to eliminate lines according to some of these measurements, they have not gained widespread acceptance. The reason appears to lie in the sensitivity of calibration to changes in variety, growing location and season, which necessitates frequent re-calibration. The availability to breeding programs of whole grain instruments provides a renewed incentive to develop the use of NIR to take the place of the entire procedure for quality screening of early generation lines where the numbers and quantity of samples preclude the use of the traditional methods. A recent development in instrumentation is the diode array spectrometer as exemplified by the Perten Model DA-7000. This type of instrument is quick to load as it requires no sample cell, and spectral data are acquired very rapidly; typically, an analytical result can be obtained in 15 s.

NIR is used in Australia to predict optimum fertilizer requirements of cereal crops by analysis of total nitrogen and carbohydrate in plant tissue samples. The tissue test for rapid determination of shoot nitrogen status in cereal crops was first developed for rice. The test has since been extended to encompass wheat shoot nitrogen and fructans. The tissue testing system is based on a plant sample taken by the farmer at a specific growth stage (Zadok scale) which is dried and analyzed by NIR. The nitrogen and fructan results are then entered into a database which is used to determine the appropriate fertilizer recommendation.[14]

The use of NIR technology to help farm managers understand the fertility of their crops and to segregate or blend grain on its protein content prior to delivery is gaining in popularity. In many countries, the price

of wheat is determined by its protein content, often with substantial price increments between grades. This has encouraged some farmers to blend wheat on farm according to its NIR protein to increase their deliverable tonnage of higher priced grain. Providing the farm's instrument has been properly calibrated and is well maintained, this strategy should be financially successful. Some farmers are also realizing that NIR has the potential to analyze a large number of samples in a short period of time which could enable them to assess their crop for optimum development or to map differences in fertility across fields.

NIR has been used in Australia, Canada, Europe and the USA to monitor growers' deliveries of wheat and barley for two decades, and some of the first research papers, e.g. Williams,[15] described the calibration of instruments for this purpose. Canada was the first grain-producing country to utilize NIR for all wheat protein testing in its segregation program. As well as increased efficiency of the Canadian wheat segregation program, the adoption of NIR testing has resulted in total cost savings to date of CAN$2.5 m. Furthermore, the replacement of the Kjeldahl system, which involves concentrated sulphuric acid and heavy metal catalyst, by the chemical-free NIR method has enabled Canada to lead the way in environmentally safe large-scale protein testing of grain. The Canadian Grain Commission now uses NIT exclusively for providing quality data in support of the marketing of about 50 million tonnes of grain annually.

By 1980, NIR protein testing had become the official method of the US Federal Grain Inspection Service (FGIS) and since then has been in continuous routine use in wheat segregation in the USA. In the USA, following the Grain Quality Improvement Act 1986, hardness measurements have been introduced as part of the wheat grading system. Wheat hardness has been measured in both meal and whole grain. Since there is no international standard reference method for wheat hardness, a definition based on NIR spectra of ground grain has been proposed by Norris et al.[16]

Delwiche et al. in the USA[17] have carried out a systematic study to develop a wheat classification system for US market class based on NIR protein and hardness measurements on single kernels. This concept is being developed by the FGIS into a practical system for the objective, rapid and sensitive detection of mixtures of wheat classes.

In Europe in the early 1980s, flour millers encountered a need to stimulate the cultivation of higher tonnages of high protein wheat by payment of premiums for protein content. A rapid method of load-by-load testing which produced results acceptable to both farmers and millers became necessary in order to calculate the price on delivery to the mill. NIR was adopted for this purpose in

Table 1 NIR applications in quality testing of wheat breeding lines

Constituent	Wheat	Flour
Protein	✓	✓
Moisture	✓	✓
Hardness	✓	
Water absorption		✓ (indicative)

a number of European countries including UK, Ireland, Germany and Sweden.

Initially, instruments based on reflectance measurements on ground grain were used for testing grain at harvest and these were shown to result in calibrations for protein which were stable over growing locations and seasons and transferable between instruments.[18] By the mid 1980s, NIT instruments had become available. These instruments typically employ a hopper, into which a sample of whole grain is placed, to dispense the sample into the measurement compartment where it is analyzed by transmittance spectroscopy (Figure 4). Usually, 4–20 subsamples are analyzed sequentially and the results averaged to obtain a more representative result for the sample. The ability to perform protein testing on whole grain eliminates the need for grinding, thus simplifying and speeding up the analysis. Protein calibrations for whole wheat and barley have been shown to perform with equal accuracy to those derived for the ground grain.[19] However, the criteria for accuracy at receival are more stringent than those for selection of breeding lines. This is because grower payments are determined according to the NIR protein content. In some countries, the payment scale is not continuous but there are cutoffs between different grades where there may be substantial price differentials. Consequently, although the accuracy of NIR calibrations are normally assessed over a wide range, in this case the scatter around a much smaller range becomes important.

In grain trading it is highly desirable to eliminate between-instrument variation in analysis results so that the farmer receives the correct grading and thus fair payment irrespective of the receival silo delivered to. This can be achieved by networking so that small differences in the response of individual instruments are corrected for in the calibration. All instruments in the network are connected to a central computer by modems, allowing simultaneous and uniform calibration and update of all instruments in the network. In 1995, all the European networks were brought together into "The European Grain Network" with the aim of standardizing NIR grain testing throughout the continent.

Paddy rice must be harvested at the optimum maturity to obtain high yield and quality. This is assessed by the moisture content of rice at receival. NIR reflectance instruments are unsuitable for use in rice receival testing because of the high moisture content at which rice is harvested. Following the successful development of NIT calibration for whole rice grain, Australian rice growers have adopted this technology for moisture testing on paddy rice.

The use of NIR technology to determine the protein and moisture contents of both wheat and flour is now routine practice in flour mills worldwide. It is used for testing each delivery of wheat in order to make decisions about acceptance, price and binning; for determination of conditioning time from measurement of hardness; and for analyzing flour to check that it complies with specifications before shipment to the customer. However, the power of NIR goes far beyond mere replacement of wet chemical analysis for raw material and product testing. With the introduction of an NIR instrument into the mill, the laboratory was able for the first time to make a direct contribution to controlling the process in real time. So useful has NIR proved to be for this purpose that in many mills it soon became moved from the laboratory into the mill itself where it could be used off-line by shift millers for process monitoring whenever the mill is running. The success of NIR for this purpose gave it the necessary credibility as a potential on-line sensor in flour mills (see section 3.3).

Many large plant bakeries employ NIR to monitor the quality of their flour and other raw ingredients. Typically, flour would be tested for protein and moisture (possibly, but less commonly, color and water absorption). In biscuit and confectionery bakeries, other ingredients such as sucrose, chocolate etc. may also be analyzed. Having verified the composition of ingredients against their quality specifications, the next point of control of a bakery process is at the dough mixing stage. Detection of faults in the dough avoids energy wastage in baking an out-of-specification product and, for biscuit doughs, may allow the dough to be re-cycled. NIR has been used for compositional analysis of intact biscuit and bread dough pieces and for monitoring the progress of bread dough mixing.

NIR calibrations for compositional analysis of short-sweet biscuit doughs enabled the simultaneous detection of errors in fat of 5% relative to the total amount of fat in the recipe and gross errors in the water, sucrose and flour percentages.[20] NIR has also been used to determine the protein and fat contents in bread dough.

During baking, the moisture content of doughs is reduced from about 18% to 2% for biscuits and 60% to 40% for bread. Monitoring the changes in the moisture content during baking allows the oven performance to be optimized. However, this is not easy because there is a non-uniform distribution of water content through both the dough and the freshly-baked product.

For biscuits, an Infrared Engineering MM55 Gauge (see section 3.3) is commonly used to monitor the moisture contents of biscuits on a conveyer as they leave the oven. This measurement depends on a reproducible relationship between the gauge reading which represents the surface moisture content in the freshly-baked biscuit and the bulk moisture content of the fully equilibrated product in packet and on strategies to compensate for the discontinuous nature of discrete products on a conveyer.

Table 2 NIR applications in the analysis of cereal products

Product	Moisture	Protein	Fat	Starch	Sugars	Fiber
Bread	✓	✓	✓	✓	✓	
Biscuits		✓			✓	
Cake mixes		✓			✓	
Breakfast cereals			✓			✓
Pasta	✓	✓	✓			
Snack foods				✓		✓

NIR is applicable to the analysis of moisture, protein, fat, starch, sugars and fiber in intact cereal foods such as bread, biscuits, cake mixes, breakfast cereals, pasta and snack foods (Table 2).

NIR has been applied to the measurement of the rate of staling of white bread crumb without the use of a reference method.[21] Rate constants were calculated directly from plots of second derivative NIR data versus time. This is a rare example in food research where NIR has been used as a primary method.

5.2 Milk and Dairy Products

Milk analyzers based on mid IR spectroscopy have been available since the 1960s but it was not until much later that NIR began to be used in the dairy industry. Although the mid IR is satisfactory for the proximate analysis of liquid milk, dairy chemists are faced with one of the widest ranges of sample types in the food industry. NIR has a key role in the analysis and process control of dairy products. It offers flexibility in the analysis of protein, moisture, fat and lactose contents in a wide range of dairy products including:

- liquid milk
- dried whole milk, skim milk and whey powders
- cream
- traditional and processed cheese.

Many of these products are emulsions whose sampling for classical chemical analysis is difficult. For example, blending such samples changes their physical characteristics. NIR offers the possibility of on-line analysis which avoids the need for batch sampling and minimizes sampling error by averaging of virtually instantaneous, continuous measurements. Milk powders are analyzed on-line using a powder analyzer which enables the moisture content to be controlled. For most other on-line applications, fiber-optic probes are used. These have the advantages of minimal maintenance owing to the absence of moving parts, robustness to high temperatures and pressures and the survival of clean-in-place protocols involving the use of caustic chemicals. Two examples of on-line NIR fiber-optic measurement in dairy processing are moisture control of cream cheese and processed cheese. Cream cheese is manufactured in a series of set tanks from which product is transferred into a separator. NIR monitoring of the product from the separator enables compensation for the variation in characteristics from different set tanks. NIR measurements of processed cheese have been used for process diagnostics in which a greater understanding of the effect of temperature on the final moisture content has been gained.

The extent of development of NIR applications in the dairy industry is such that it is possible to purchase an instrument which is factory-calibrated for proximate analysis of cheddar cheese. However, these calibrations are only valid for cheddar made to the traditional recipe and sampled directly from the vat; non-traditional and matured cheddars require different calibrations.

5.3 Meat

NIR spectroscopy is widely used in the meat industry for proximate analysis. A special interactance fiber-optic probe has been designed to spear carcasses and determine their fat content. This enables the carcasses to be sorted prior to butchering.

Dedicated instruments are available to determine the protein, fat and moisture contents of ground meat and meat products and factory-set calibrations are available for cooked meat, cooked ham, liver sausage, and pepperoni. Meat samples are minced then blended in a food processor before being packed into an open sample cell. The amount of sample needs to be controlled either by mass or by depth.

Isaksson et al.[22] have used the MM55 on-line gauge (see section 3.3) for proximate analysis of ground beef directly at a meat grinder outlet. NIR has also been used for sensory analysis of sausages and to discriminate between fresh and frozen-then-thawed beef.

5.4 Fish

The analysis of farmed atlantic salmon and rainbow trout enables the optimization of their processing. NIR fiber-optic interactance probes can be used to measure nondestructively the protein, moisture and oil contents of whole fish.[23]

5.5 Fruit and Vegetables

Fresh fruit and vegetables are graded by shape, size and color. Objective, nondestructive methods of sorting enable growers and packers to market a consistent product over an extended season. In addition, high-quality produce can be segregated for high-return markets while

lower-quality produce can be identified for processing or other uses.

The nondestructive sorting of fruit for ripeness by optical spectroscopy was originally carried out in the visible region. However, the availability of fiber-optic interactance probes led to a resurgence of research interest in this application via the determination of sugar content. The reason for developing a direct measure of sugar content of fruit is that appearance is not a reliable guide to sweetness. Japanese researchers[24,25] have demonstrated the successful application of NIR to the on-line determination of sugar content in intact peaches and mandarins and developed an automated fruit sorting machine based on this principle. Australian scientists have extended this concept to tropical fruits such as melons, mangoes and pineapples.

5.6 Confectionery

NIR applications in the confectionery industry include the determination of moisture in granulated sugar and chocolate crumb, protein and oil in cocoa powder and fat in whole chocolate. NIR data have also been shown to correlate highly with sensory data on raw and roasted cocoa beans, chocolate mass and finished chocolate.

5.7 Beverages

When barley is germinated under controlled conditions, it undergoes a series of complex biochemical reactions which result in its conversion to malt. The malt is then mashed with water to produce a liquid called wort which is then fermented to produce beer. Since malting takes about twelve days, barley breeders need a rapid means of predicting the malting quality of barley grain. Calibrations have been developed for nitrogen, lysine, β-glucan and malt hot water extract. As with wheat functional quality, NIR analysis of barley is used to predict the quality of material which is derived by processing of the grain and there is doubt whether tests on ungerminated grain fully account for the interaction of enzymes and substrates during germination. Attempts have been made to improve the accuracy of prediction by analysis of key quality characteristics of the malt or the wort (Table 3).

In the brewing industry, NIR is widely used to monitor the original gravity and alcohol content of beer using on-line flow-through cells. Standard errors of the calibration for alcohol of $0.1 - 0.2\%$ have been reported[2] using either transmittance or transflectance cells. The Liquidata probe has been used to achieve a process guarantee on any beer to better than $\pm 0.04\%$ alcohol.

NIR is used to monitor fruit quality and determine the alcohol content of wine and dedicated filter instruments for wine analysis are commercially available.

Table 3 NIR applications in quality testing of barley breeding lines

Constituent	Barley	Malt	Wort
Total nitrogen	✓	✓	
Moisture	✓	✓	
Lysine	✓		
β-Glucan	✓		
Extract	✓	✓	✓
Soluble protein			✓
Free α-amino N			✓

Other beverage applications include the moisture content of tea and coffee, sugar content of fruit juices and the sensory quality of tea and coffee blends.

5.8 Authenticity

The study of food authenticity involves establishing whether a sample is genuine in terms of its description, including geographical origin. Many analytical techniques have been used to examine the authenticity of a wide range of different foods. The applications of NIR for authenticity testing of coffee, fruit pulps, milk powders, orange juice, pig carcasses, rice, sausages, sugars, vegetable oils, wheat grain and wheatflour have been reviewed by Downey.[26] These applications are based on the principles of discriminant analysis (see section 4.4) in which the problem is to compare the spectrum of the test sample with a reference library (see section 4.2). The library should contain examples of known authentic and adulterated samples. In the simplest case, the question is simply whether the test sample belongs to one of two populations such as ''Basmati rice'' or ''not Basmati rice''. Often, however, it is required to classify within a series of possible classes (e.g. different vegetable oils or wheat varieties), to identify a particular kind of adulteration (e.g. medium invert beet sugar, pulp wash or zest oil in orange juice) or to quantify an adulteration (e.g. non-dairy fat in milk products).

6 THE LITERATURE

Scientific papers describing the practical applications of NIR in food analysis are spread widely throughout the literature. As evidenced by the key references cited in this article, such papers may be found in journals devoted to analysis, spectroscopy, general food science or more specialized areas of food science. This makes it particularly difficult to search the literature. However, *NIR News* (NIR Publications, Chichester, UK) has since

1990 included a references section in an attempt to overcome this problem.

The only scientific journal devoted exclusively to NIR spectroscopy is *Journal of Near Infrared Spectroscopy* (NIR Publications, Chichester, UK). Volume 4 was dedicated to Karl H Norris, the founder of modern NIR analysis. It contains historical, review or leading edge papers by many of the pioneers of NIR spectroscopy and provides a particularly valuable introduction to the technique.

The proceedings of the series of international conferences on near-infrared spectroscopy (ICNIRS) provide a valuable addition to the literature on the developments in and applications of NIR spectroscopy:

- J. Hollo, K. J. Kaffka, J. L. Gonczy, *Near-Infrared Diffuse Reflectance/Transmittance Spectroscopy*, Akademiai Kiado, Budapest, 1987.
- C. S. Creaser, A. M. C. Davies, *Analytical Applications of Spectroscopy*, Royal Society of Chemistry, Cambridge, 1988.
- M. Iwamato, S. Kawano, *Proceedings of the Second International Near-Infrared Spectroscopy Conference*, Korin, Tokyo, 1989.
- R. Biston, N. Bartiaux-Thill, *Proceedings of the Third International Conference on Near-Infrared Spectroscopy*, Agricultural Research Centre Publishing, Gembloux, Belgium, 1990.
- K. I. Hildrum, T. Isakksson, T. Naes, A. Tandberg, *Near-Infrared Spectroscopy. Bridging the Gap between Data Analysis and NIR Applications*, Ellis Horwood, Chichester, 1992.
- G. D. Batten, P. C. Flinn, L. A. Welsh, A. B. Blakeney, *Leaping Ahead with Near-Infrared Spectroscopy*, Royal Australian Chemical Institute, Melbourne, 1995.
- A. M. C. Davies, P. C. Williams, *Near-Infrared Spectroscopy: The Future Waves*, NIR Publications, Chichester, 1995.

There are only two books currently in print devoted to NIR analysis:

- D. A. Burns, E. W. Ciurczak, *Handbook of Near-Infrared Analysis*, Marcel Dekker Inc., New York, 1992.
- B. G. Osborne, T. Fearn, P. H. Hindle, *Practical NIR Spectroscopy*, 2nd edition, Longman, Harlow, 1993.

7 STANDARD METHODS

The following standard methods of NIR analysis of foods have been adopted:

American Association of Cereal Chemists (AACC)

Method 39-01. General near-infrared instrumentation and techniques. First Approved: 1989.

Method 39-10. Near-infrared reflectance method for protein determination. First Approved: 1982.

Method 39-11. Near-infrared reflectance method for protein – wheat flour. First Approved: 1985.

Method 39-20. Near-infrared reflectance method for protein and oil determination – soybeans. First approved: 1991.

Method 39-21. Near-infrared method for whole-grain analysis. First Approved: 1991.

Method 39-25. Near-infrared method for protein content in whole-grain wheat. First Approved: 1995.

Method 39-70A. Wheat hardness as determined by near-infrared reflectance. First Approved 1986.

Association of Official Analytical Chemists (AOAC)

Official First Action Method 997.06. Protein (crude) in wheat. Whole grain analysis, Near-infrared spectroscopic method. Adopted: 1997.

International Association for Cereal Science & Technology (ICC)

ICC Standard No. 159. Determination of protein by near-infrared reflectance (NIR) spectroscopy. Approved: 1995.

ICC Recommendation No. 202. Procedure for near-infrared (NIR) reflectance analysis of ground wheat and milled wheat products. Approved: 1986.

Royal Australian Chemical Institute (RACI) Cereal Chemistry Division

Method No. 11.01 Determination of protein and moisture in whole wheat and barley by NIR. First Approved: 1998.

ACKNOWLEDGMENTS

ABBREVIATIONS AND ACRONYMS

AACC	American Association of Cereal Chemists
ANN	Artificial Neural Network
AOAC	Association of Official Analytical Chemists
AOTF	Acousto-optically Tunable Filter
FGIS	Federal Grain Inspection Service
ICC	International Association for Cereal Science & Technology
ICNIRS	International Conferences on Near-Infrared Spectroscopy

IR	Infrared
NIR	Near-Infrared
NIROS	Near-Infrared On-line Powder Sampler
NIT	Near-Infrared Transmittance
PLS	Partial Least Squares
RACI	Royal Australian Chemical Institute

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