Applications of Near Infrared Spectroscopic Analysis in the Food Industry and Research

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Abstract

Near infrared spectroscopy (NIR) is routinely used in agriculture, for the analysis of pharmaceuticals, combustion products, astronomy, food stuffs and in research. The method has been broadly accepted and is widely used in quality assessment of foods, beverages and in food and beverage research. The method is relatively inexpensive, rapid, preserves the sample and is able to measure several constituents concurrently. Current food and beverage applications of NIR are dominated by proximate quality assessment, and research focused on this aspect of near infrared analysis is very active. Interest in the application of near infrared spectroscopy to microbiological research and analysis has grown in recent times. However, the use of NIR as a tool for microbiological monitoring, ecological and physiological research remains underdeveloped. Research investigating its use in these and other microbiological monitoring functions is required and would provide a valuable, cost effective addition to proximate systems already in place within many food industries.

Introduction to molecular spectroscopy

Molecular spectroscopy involves the analysis and quantification of molecular responses to introduced signals of known energy or frequency. All molecules (e.g. water, glucose, protein) have a defined amount of energy. Briefly, when alternate energy (e.g. infrared radiation) is introduced, an energy exchange occurs between the introduced energy and the energy contained within the molecule. In simplest terms, this is expressed as absorbance (energy is absorbed resulting in a loss of introduced energy), attenuated (energy is scattered resulting in a loss of introduced energy) or emitted (energy is released resulting in a gain on the introduced energy). As individual molecules and molecular groups (e.g. alcohols, nucleic acids, proteins, sugars and fats) have defined energy, by applying external energy of a known amount/type, we can structurally identify, quantify and even determine the natural state of these molecules within complex samples and mixtures.

Molecular spectroscopy encompasses a broad range of physical, inorganic, organic and biochemical scientific methods, many of which are beyond the scope of this report. However, in order to properly introduce the concept of near-infrared spectroscopy (NIR), the *generalised* principle of electromagnetic spectroscopy, the broad group of molecular spectroscopic methods within which NIR belongs, must first be covered.

Electromagnetic spectroscopy analyses and measures the electromagnetic spectra absorbed or released by given atoms, molecules and molecular groups. These spectra are waves of energy of defined frequencies (measured in Hertz), and subsequently wavelength (measured in metres), which are characteristic for individual molecular groups, molecules and atoms (Figure 1). The infrared region of the electromagnetic spectrum may be divided both instrumentally and functionally into near, middle and far infrared spectra. Near infrared (NIR) spectroscopy corresponds to a wavelength range of between 700 and 2500nm on the visible light side of the divided infrared spectrum (Figure 2).

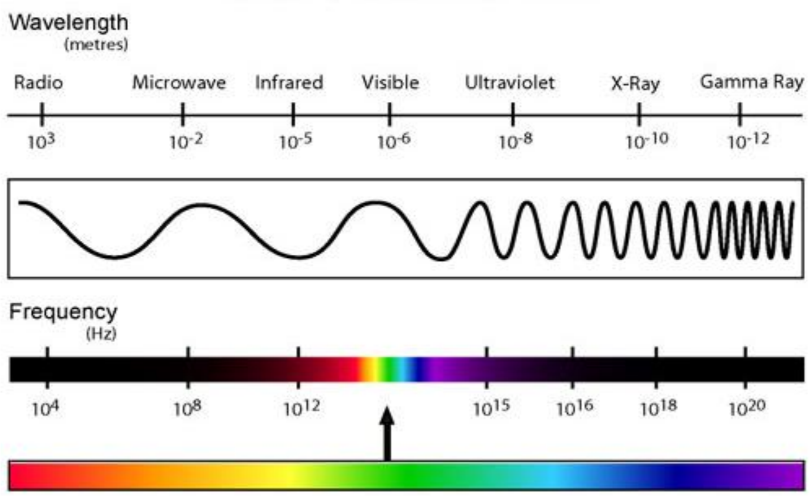

Figure 1. The electromagnetic spectrum highlighting the relationship between increased frequency and decreased wavelength. The region of the spectrum we perceive as visible light corresponds to the range of 10^{-3} m (short wavelength edge of the microwave band) and 10^{-8} m (long wavelength edge of the ultraviolet band). Individual substances, molecular groups, molecules and atoms absorb or emit energy at characteristic regions of this spectrum. Image sourced from www.wordpress.com (http://mihneaboiangiu.wordpress.com/2009/04/17/relativ/).

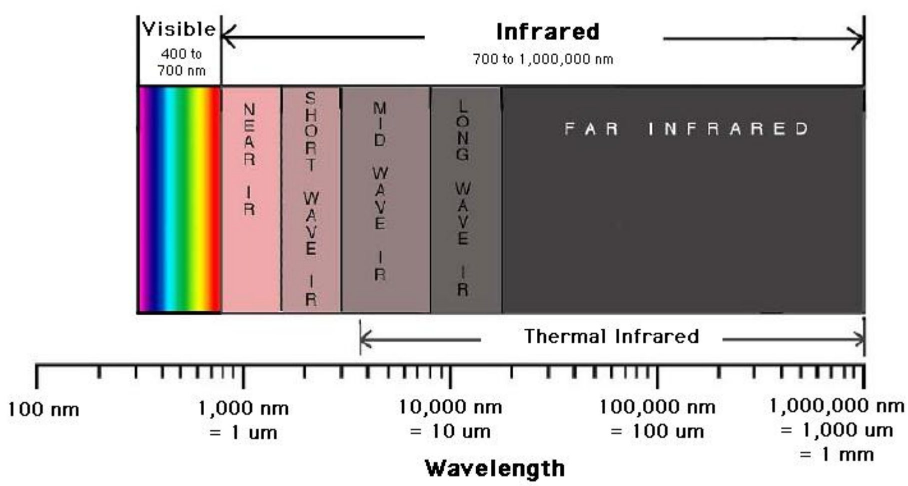

Figure 2. Divisions of the infrared electromagnetic spectrum. The near infrared region corresponds to a wavelength range of between 700 and 2500nm. The mid-infrared region is further divided into short, mid and long wave infrared and corresponds to a wavelength range of $2500 - 5 \times 10^4$nm. Finally the far infrared region corresponds to a wavelength range of $5 \times 10^4 - 1 \times 10^6$nm. Image sourced from “Measuring Vegetation Health” (mvh.sr.unh.edu/mvhtools/near_ir.htm).

Applying infrared energy to a substance will cause the molecules within that substance to undergo wavelength specific transitions. In simplest terms, application of far infrared energy will cause rotation of bonds within given molecules and middle infrared will cause fundamental vibrations. Near infrared energy gives rise to overlapping overtones and combinations of rotation and vibration of C – H, O – H and N – H chemical bonds. Overtones are the result of ≥2 molecular absorptions of energy quantum. As a quantum of energy is applied to a given molecule, the molecule absorbs that energy, and vibration corresponding to a simple “near” harmonic motion is produced (e.g. wave). The overtone band of this simple harmonic motion has approximately twice the frequency of the principle vibrational frequency. The intensity of the overtone band is dictated by the level of “anharmonicity” of the principle vibrational band. Chemical bonds that vibrate with large amplitude (e.g. hydrogen bonds) have a high degree of anharmonicity. These bonds are very common and as a consequence, can dominate the observable NIR bands. As such, molecular overtones and combinational vibrations characteristic of NIR produce very broad, complex, overlapping spectral outputs that can conceal *specific* information on chemical assignments. This is overcome through the use of multivariate calibration, chemometric techniques and multivariate methods such as principle component analysis and partial least squares (Koljonen *et. al.* 2008; Cozzolino *et. al.* 2009; Gishen *et. al.* 2005) (Figure 3.).

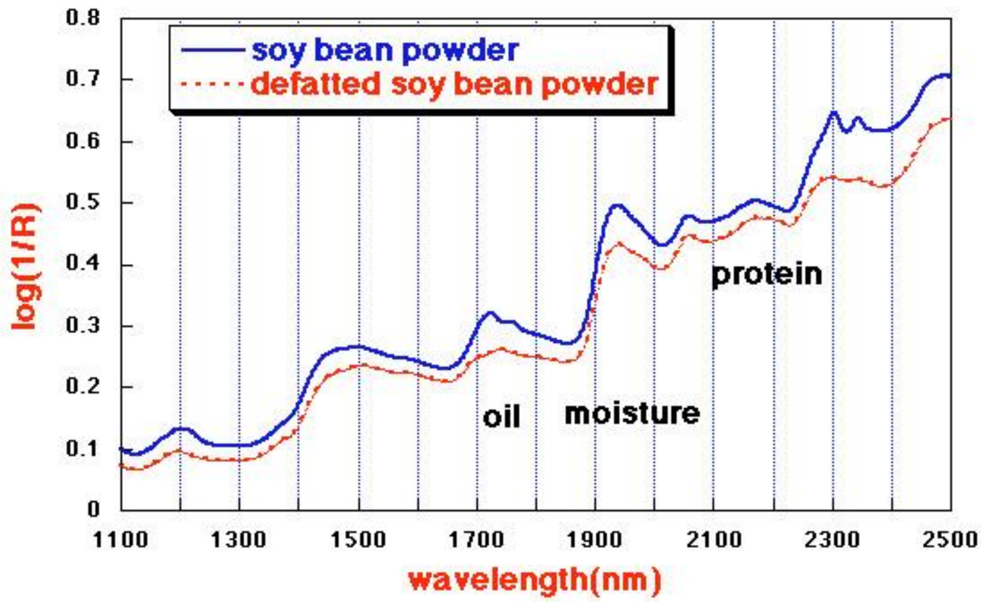

Figure 3. Simple infrared spectral output differentiating full fat soy-bean powder from defatted soybean powder. The output is made up of broad overlapping spectra that, without chemometrics, conceals specific details including broad categorisation of major groups. Image sourced from "The Near Infrared Method" (konarc.naro.affrc.go.jp/.../jouseki/ad07-e.html).

Multivariate calibration remains the most important step for reliable and accurate NIR analysis. It is aimed at relating the observed spectra to a property of interest. This must be selected empirically based on sample type and is primarily targeted at increasing resolution of overlapping bands and deconvolution of background signal. Burns and Ciurczak (2007) provide an excellent overview of this component of analysis. As a result, depending on the combination of molecules, the ability to physically undergo transitions, and application of multivariate calibration; characteristic spectra can be acquired and compared for individual combinations of molecules creating a "finger print" or "model" of organisms, individual substances and complex mixtures.

Food industry and research applications

Applications of NIR spectroscopy are varied and it is now routinely used in agriculture, for the analysis of pharmaceuticals, combustion products, astronomy, food stuffs and in research. The method has been broadly accepted and is widely used in quality assessment of foods, beverages and in food and beverage research. A range of analytical laboratories throughout the world now offer a variety of NIR spectroscopic services and products targeted at a range of applications (see *Additional Resources*).

Advantages of near infrared spectroscopy include minimal sample preparation (may be performed *in situ* in many instances), rapid analysis, sample preservation, and much deeper sample penetration than far or mid infrared radiation. Furthermore, NIR analysis permits measurement of several constituents concurrently. As a result, NIR analysis remains relatively inexpensive when compared to equivalent technologies. There are little or no specialised sample preparations involved; externally performed analysis costs as little as $5 AUS per sample at the time of this writing[1], increasing the appeal and broadening the potential use of this analytical tool.

Current food and beverage applications of NIR spectroscopy are dominated by quality assessment. This is both qualitative and quantitative. Near infrared spectroscopic qualitative assessment can provide information on the consistency (or presence) of both raw materials and end product. Quantitative NIR assessment is used to measure the level of specific components within complex mixtures *in situ*, thereby minimising sample preparation, saving time and reducing costs.

[1] Quote obtained from Central Science Laboratory, University of Tasmania, Australia on 10th July, 2009. Cost includes equipment use ($4), all consumables ($1) and preliminary data analysis. User performed data analysis is required following initial training, calibration and optimisation. Subject to change.

Near infrared spectroscopy is widely used in the meat, dairy and seafood industries for proximate analysis. This is aimed predominantly at determining fat / oil, protein and moisture levels in the associated products, serving a grading and quality assurance function. An excellent review on the application of NIR to food and beverage assessment over the past decade was recently compiled by Woodcock and colleagues (2008). Research focused on this aspect of near infrared analysis is very active, with particularly emphasis on quantitative and chemometric aspects. The Journal of Near Infrared Spectroscopy (IM Publications, United Kingdom) (http://www.impublications.com/nir/jtoc/16_6) provides a thorough overview of current research in near infrared spectroscopy. In addition to this, the publishers host an NIR discussion forum (http://www.nirpublications.co.uk/cgi-bin/discus/discus.cgi), NIR software archive (http://www.impublications.com/nir/page/software), and many other resources aimed at communicating information on near infrared research and industry applications.

Interest in the application of near infrared spectroscopy to microbiological research and analysis has grown in recent times with a number of organisations focusing on the technique. This has largely been driven by advances in chemometric analysis, analytical software, and recognition of the many advantages this technique provides over similar methods. A variety of studies investigating the potential of NIR for rapid detection and enumeration of microbes, including within food samples, have been conducted (Saranwong and Kawano, 2008; Rodriguez-Saona *et. al.* 2001). Many of these studies have involved direct detection and enumeration, however works indirectly assessing microbial populations through detection of metabolites (by-products of metabolism) have been performed (Cozzolino *et. al.* 2006). The application of NIR to metabolomic analyses presents an exciting opportunity for relatively simple, low cost, advanced studies of the physiology of microbial populations.

Research at the University of Tasmania's Food Safety Centre

Researchers at the University of Tasmania's Food Safety Centre are investigating the use of NIR spectroscopy as a tool for studying microbial physiology and ecology. In the microbial physiology component, NIR will be used to measure metabolite production and nutrient consumption by the food-borne pathogen *Listeria monocytogenes* under environmental stress conditions (such as nutrient and pH flux) simulating those found within food production and processing environments. This is targeted at indirectly identifying differential species and strain responses to these stresses by comparing how nutrients are consumed and metabolites produced. In separate microbial ecology studies, NIR spectroscopy will be used as a rapid tool for microbial community profiling on food matrices, such as modified packaged raw fish. Ultimately it is hoped that information derived from both of these works could be used to guide the logic of intervention and prevention strategies against food-borne pathogens within food systems environments.

Conclusion

Near infrared spectroscopy is a cost-effective, rapid analytical tool with potential beyond its current use within food industry. As advances in chemometric analysis and analytical software develop, calibration and multivariate methods will follow allowing more information to be retrieved from the complex spectra, broadening the scope of this analytical tool.

At present, the use of NIR as a tool for microbiological monitoring, ecological and physiological research remains underdeveloped. The technique shows great promise for these applications. Examples include:

- *In vitro* and *in situ* analysis of biofilm formation, structure and development by food-borne pathogens. Biofilm formation leads to persistent microbial contamination of food processing establishments. Studies of microbial biofilms can be complex and it can be difficult to accurately depict those found within food factory environments. Detailed real-time knowledge of the components of biofilm complexes could allow identification of weaknesses within these biological systems and provide potential "industry relevant" targets for their control.

- A *rapid* means of profiling microbial communities present in food products. Knowledge of the microbial communities associated with contamination and spoilage of foods, including succession throughout the processing chain, could significantly contribute to the safety of end product and minimise avoidable product loss and the associated costs.

- Development of food-borne pathogen spectra libraries. Metabolomic profiling of food-borne pathogens under optimal and challenging environmental conditions could provide NIR spectral libraries that contain indirect markers for both pathogen presence and physiological state. Aside from the presence or absence of food-borne pathogens, these markers could provide information that allows assessment of how microbes are *responding* to the conditions present on/in the food product/environment. This could aid in the assessment of food safety/preservation systems already in place, and guide implementation of intervention and preventative strategies based on the observed physiological responses of the resident and detected microflora.

Researchers at the University of Tasmania's Food Safety Centre are evaluating the use of NIR for the development of methods and acquisition of knowledge that can readily be converted to tangible outcomes for food industry. Considering NIR spectroscopic analysis already forms a routine component of many food industries, research investigating its use in a range of microbiological monitoring functions would provide a valuable addition to proximate systems that are already in place, at little or no extra cost.

Additional Resources

Examples of analytical laboratories, product distributors and consultancies providing near infrared spectroscopic services:

1. Central Science Laboratory, University of Tasmania

Provides a range of electromagnetic spectroscopic analytical services.

http://fcms.its.utas.edu.au/csl/csl/facilitiesdetails.asp?lFacilityId=270

2. The Near Infrared Research Corporation

Provides assistance and products associated with near infrared spectroscopic analysis and associated chemometrics.

http://www.nearinfrared.com/

3. Cognis Agrosolutions

Provider of on-site infrared analysis.

http://www.cognis.com/products/Business+Units/AgroSolutions/Grain+Analysis/

4. Cromar Solutions Ltd.

A near infrared spectroscopy consultancy service.

http://www.cromarsolutions.co.uk/infrared.htm

5. Buchi Laboratory Equipment

A near infrared spectroscopy consultancy service. Provider of N.I.R hardware, software and spectra reference libraries.

http://www.buchi.com/NIRSolutions.72.0.html?utm_id=niso&gclid=CL69ksWAiZwCFRIcawody3JxYA

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