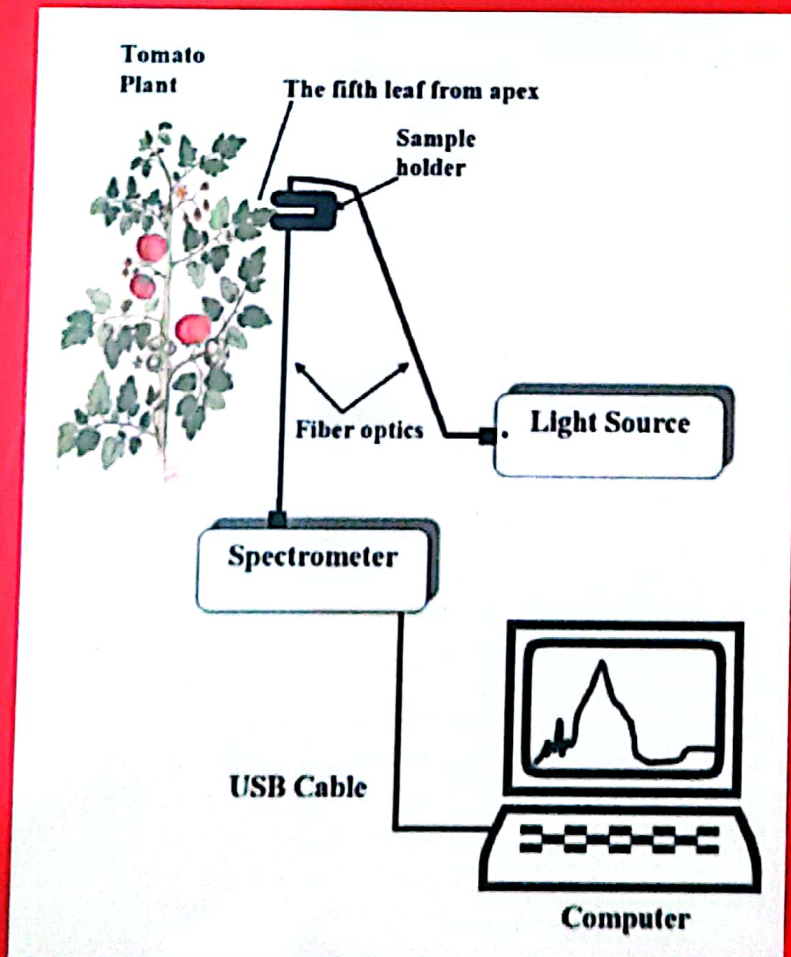


The Application of Near Infrared Spectroscopy and Chemometrics

for Monitoring Water Stress in Tomato Plants



Dr. Agr. Sc. Diding Suhandy, S.T.P., M.Agr.

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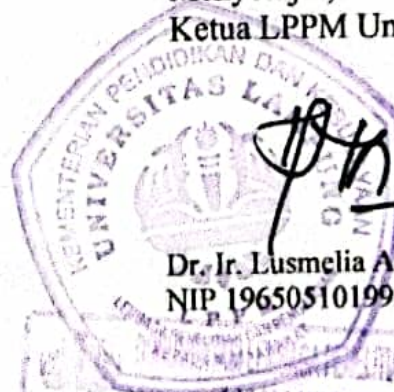
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Preface from Author

This book is part of master thesis which has been submitted to Kochi University, Japan to get master degree in Agriculture (M.Agr). The author stayed in Kochi University in the period of 2004-2006 under AAP (Asia, Africa and pan Pacific) program. The author would like to extend his grateful thanks to the Technological and Professional Skills Development Sector Project (TPSDP)-Indonesian Ministry of National Education for the scholarship grant. Special thanks are due to the cooperation of the all member of SPMU (Sub Project Management Unit) Lampung University who managed the scholarship program.

This book has 7 chapters including Introduction, Near Infrared Technology, Water Stress Measurement in Plants, Determination of Leaf Water Potential in Tomato Plants Using NIR Spectroscopy, Developing a Calibration Model of Leaf Water Potential Determination Using NIR Spectroscopy with Temperature Compensation, On-plant Monitoring of Leaf Water Potential Measurement Using NIR Spectroscopy for Water Stress Management and finally General Conclusion. Some of these chapters have been published in reputable journals. However, in order to construct a comprehensive story about LWP determination for water stress management, we decide to publish the master thesis into a reference book.

The author wishes to express his sincere gratitude to Professor Takahisa Matsuoka, the major advisor, for his outstanding academic guidance and continuous encouragement during the master program in Kochi University and in the preparation of this thesis. The author also would like thanking to Professor Katsumi Ishikawa (Kochi

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INTRODUCTION

1.1. Background

Recently water stress has been used as a promising method for improving the quality of several fruits and vegetables. In Japan, under protected cultivation either in plastic greenhouses or glasshouses, most of fruits and vegetables cultivation especially tomatoes are grown under water stress condition. The water stress is induced artificially by withholding irrigation in several times (intermittent irrigation) or by increasing the salinity or the fertilizer concentration of nutrient solution (high Electric Conductivity/EC).

A tomato fruit growing under water stress condition was high in total soluble solid contents (SSC), sugar and acid contents (Mitchell *et al.*, 1991a; Petersen *et al.*, 1998). Recent works by Nuruddin *et al.* (2003) and Flores *et al.* (2003) demonstrated similar results. However, the total yield was decreased due to weight and size reduction and reducing marketable yield in form of high blossom-end rot (BER) incidence and small fruits (Ho *et al.*, 1987; Pulupol *et al.*, 1996; Cuartero and Fernandez-Munoz, 1999). The yield reduction was mainly caused by decreasing of average size fruits (Okano *et al.*, 2002; Navarro *et al.*, 2005) or reducing average weight (Cuartero and Fernandez-Munoz, 1999; Flores *et al.*, 2003). The occurrence of BER was mainly caused by inhibition of Ca^{2+} uptake because of the water stress application (Saure, 2001; Navarro *et al.*, 2005).

The degree of yield reduction was depending on the degree of water stress (Mirzahi *et al.*, 1988; Mitchell *et al.*, 1991b). For example salinity with 30 mM NaCl (moderate water stress) reduced the average tomato weight by 20%, whilst 60 mM NaCl reduced the average tomato weight by 42% (Flores *et al.*, 2003). Hence, the proper of water stress could be obtained if we can maintain the optimum level of water stress. It can be done only if we can monitor continuously the level of water stress. For this purpose, a new method is highly required for measuring water stress in a rapid and nondestructive method. By using this new method, a real time evaluation of the water stress level would be possible.

The water stress affects the transport of water to the fruits. Several works found that the increasing of soluble solid contents and sugar content mainly caused by inhibition of water to the fruits rather than the increasing of dry matter content (Ehret and Ho, 1986; Mitchell *et al.*, 1991b; Guichard *et al.*, 2001). As a result, for the monitoring of water stress level, it is important to use a parameter directly associated with the water transport in plants.

Leaf water potential (LWP) has been widely used as one popular parameter to quantify the water deficits in leaf tissues. Under water stress condition, the LWP was highly correlated with physiological parameters of plant such as stomatal conductance and photosynthetic rate (Boyer, 1970; Gomes *et al.*, 2004). When the water stress is induced, the LWP, the stomatal conductance and the photosynthetic rate decrease. The LWP was also correlated to the leaf rolling, a simple appearance of water stress (O'toole and Cruz, 1980). For this reason, the parameter of LWP can be considered as a good indicator of water stress and monitoring this LWP values is valuable in order to maintain an optimum level of the water stress.

Nondestructive detection of water stress has become a popular field and has received considerable attention for past a decade. Several works has been conducted in order to find out a new method to detect and monitor the water stress in several plants nondestructively. Some studies used near infrared (NIR) wavelengths both in remote and close

distance to detect the water stress in corn and spinach (Jones *et al.*, 2004), in wheat (Graeff, 2004), in soybean (Gomide *et al.*, 2003), and tomato fruits (Okamura *et al.*, 2002). Kacira *et al.* (2002) used the machine vision technology to detect the water stress in New Guinea Impatiens. The other used acoustic emission (AE) technique to detect the water stress in tomato plants (Qiu *et al.*, 2002).

1.2. Water stress detection using near infrared (NIR) wavelengths

NIR wavelengths have been widely used to detect the water stress in plants. The NIR spectra both in short and long wavelength had been successfully detected the water stress in several plants. The good result of the water stress detection using NIR wavelengths can be clearly understood since that the water absorption band center at 760 nm, 970 nm, 1200 nm, 1450 nm, 1780 nm and 1940 nm (Ben-Gera and Norris, 1968; Curran, 1989). However, most of the water stress detection using near infrared spectra was employed in canopies from a remote distance. As reported by Bowman (1989) and Hunt and Rock (1989), the detection of water stress from a remote distance would be difficult because of environmental influences. From a close distance, application of NIR wavelengths has been conducted in order to detect the water stress in tomato plants (Okamura *et al.*, 2001) and the water status of several crops (Inoue *et al.*, 1993). So far, no studies reported about the LWP determination in tomato plants using NIR spectroscopy and its usage for the water stress management.

1.3. The objectives of study and book organization

The objective of this study was to develop a new method of on plant LWP determination using NIR spectroscopy and its usage for the water stress management. The study included the use of the Partial Least Squares (PLS) regression method to develop a calibration model for nondestructive LWP determination. The calibration model, which is combined with an 'on plant' NIR measurement, could be used as tool for monitoring the LWP values in the field and has a promising method for a real time water stress evaluation. To realize this purpose, three stages of study were done.

In the first study, the potentiality of NIR spectroscopy to quantify the LWP values in tomato plants was investigated. In this study a relationship between NIR spectra of tomato leaf and the LWP was investigated and then a calibration model for the LWP determination was developed. This work is described in Chapter 4. To apply a water stress management, it was required to perform an 'on plant' NIR measurement for the LWP determination in the field. For this purpose, in the next study it was highly required to develop a calibration model that could compensate the effect of temperature due to environmental changes. This work included the use of growth chamber for temperature equilibration. This is discussed in Chapter 5. Finally, using the calibration model resulted in the previous study; a monitoring of LWP values in tomato plants was evaluated. Chapter 6 is devoted to discuss this final study. Chapter 7 completes the book. It is devoted to discuss the general discussion and conclusions.

References

- Ben-Gera, I., Norris, K.H. 1968. Determination of moisture content in soybeans by direct spectrophotometry. *Isr. J. Agric. Res.* 18: 124–132.
- Bowman, W.D. 1989. The relationship between leaf water status, gas exchange, and spectral reflectance in cotton leaves. *Remote Sens. Environ.* 30: 249–255.
- Boyer, J.S. 1970. Differing sensitivity of photosynthesis to low water potentials in corn and soybean. *Plant Physiol.* 46: 236–239.
- Cuartero, J., Fernandez-Munoz, R. 1999. Tomato and salinity. *Sci. Hortic.* 78: 83–125.
- Curran, P.J. 1989. Remote sensing of foliar chemistry. *Remote Sens. Environ.* 30: 271–278.
- Ehret, D.L., Ho, L.C. 1986. Effect of salinity on dry matter partitioning and growth in tomato grown in nutrient film culture. *J. Hort. Sci.* 61: 361–367.
- Flores, P., Navarro, J.M., Carvajal, M., Cerda, A., Martinez, V. 2003. Tomato yield and quality as affected by nitrogen source and salinity. *Agronomie* 23: 249–256.
- Gomes, M.M.A., Lagôa A.M.M.A., Medina, C.L., Machado, E.C., Machado, M.A. 2004. Interactions between leaf water potential, stomatal conductance and abscisic acid content of orange trees submitted to drought stress *Braz. J. Plant Physiol.* 16(3): 155–161.
- Gomide, R.L., Tian, L., Pinto, F.A.C. 2003. Thermal and color near infrared spectral remotely sensed scanners to detect in-field soybean and corn water stress variability. *ASAE Paper No. 033127.* St. Joseph, Mich.: ASAE.
- Graeff, S. 2004. Use of reflectance measurements to clearly identify water stress in wheat (*Triticum aestivum* L.). In *Proc. 4th Int'l.*

Crop Sci. Congress., Brisbane, Australia.

- Guichard, S., Bertin, N., Leonardi, C., Gary, C. 2001. Tomato fruit quality in relation to water and carbon fluxes. *Agronomie*. 21: 385-392.
- Ho, L.C., Grange, R.I., Picken, A.J. 1987. An analysis of the accumulation of water and dry matter in tomato fruit. *Plant Cell Environ.* 10: 157-162.
- Hunt, E.R., Jr., Rock, B.N. 1989. Detection of changes in leaf water content using near-and middle infrared reflectance. *Remote. Sens. Environ.* 30: 43-54.
- Inoue, Y., Morinaga, S., Shibayama, M. 1993. Nondestructive estimation of water status of intact crop leaves based on spectral reflectance measurements. *Jpn. J. Crop. Sci.* 62: 462-469.
- Jones, C.L., Weckler, P.R., Maness, N.O., Stone, M.L., Jayasekara, R. 2004. Estimating water stress in plants using hyperspectral sensing. ASAE Paper No. 043065. St. Joseph, Mich.: ASAE.
- Kacira, M., Ling, P.P., Short, T.H. 2002. Machine vision extracted plant movement for early detection of plant water stress. *Trans. ASAE* 45(4): 1147-1153.
- Mitchell, J.P., C. Shennan, S.R. Grattan, D.M. May. 1991a. Tomato fruit yield and quality under water deficits and salinity. *J. Amer. Soc. Hort. Sci.* 116: 215-221.
- Mitchell, J.P., C. Shennan, S.R. Grattan. 1991b. Developmental changes in tomato fruit composition in response to water deficit and salinity. *Physiol. Plant.* 83: 177-185.
- Mizrahi Y, Taleisnik E, Kagan-Zuh V, Zohar Y, Offenbach R, Matan E, Colan R. 1988. A saline irrigation regime for improving tomato fruit quality without reducing yield. *J. Amer. Soc. Hort. Sci.* 113: 202-205.

- Navarro, J.M., Flores, P., Carvajal, M., Martinez, V. 2005. Changes in quality and yield of tomato fruit with ammonium, bicarbonate and calcium fertilization under saline conditions. *J. Hortic. Sci. Biotech.* 80: 351–357.
- Nuruddin, M. Md., Madramootoo, C. A., Dodds, G.T. 2003. Effects of water stress at different growth stages on greenhouse tomato yield and quality. *HortScience* 38: 1389–1393.
- O`toole, J.C., Cruz, R.T. 1980. Response of leaf water potential, stomatal resistance, and leaf rolling to water stress. *Plant Physiol.* 65: 428–432.
- Okamura, N.K., Shimomachi, T., Takemasa, T., Takakura, T. 2001. Nondestructive detection of water stress in tomato plants by NIR spectroscopy. *Environ. Control in Biol.* 39(2): 75–85.
- Okano, K., Nakano, Y., Watanabe, S., Ikeda, T. 2002. Control of fruit quality by salinity stress at various fruit development stages of single-truss tomato grown in hydroponics. *Environ. Control in Biol.* 40: 375–382.
- Petersen, K.K., Willumsen J., Kaack K. 1998. Composition and taste of tomatoes as affected by increased salinity and different salinity sources. *J. Hortic. Sci. Biotech.* 73: 205–215.
- Pulupol, L.U., Behboudian, M.H., and Fisher, K.J. 1996. Growth, yield and postharvest attributes of glasshouse tomatoes produced under deficit irrigation. *HortScience.* 31: 926–929.
- Qiu, G.Y., Okushima, L., Sase, S., Lee, I.B. 2002. Acoustic emissions in tomato plants under water stress conditions. *JARQ.* 36(2): 103–109.
- Saure, M.C. 2001. Blossom-end rot of tomato (*Lycopersicon esculentum* Mill.) - a calcium- or a stress-related disorder?. *Sci. Hortic.* 90: 193–208.

NEAR INFRARED TECHNOLOGY

2.1. Introduction

The overall objective of Near Infrared Technology is to probe a sample in order to acquire qualitative and/or quantitative information coming from the interaction of near infrared electromagnetic waves with its constituents. The analytical method resulting from the use of the NIR spectroscopic region reflect its most significant characteristic such as: fast (one minute or less per sample), nondestructive, non-invasive, with high penetration of the probing radiation beam, suitable for in-line use, nearly universal application (any molecule containing C-H, N-H, S-H, or O-H bonds), with minimum or no sample preparation demands. The combination of these characteristics with instrumental control and data treatment (chemometrics) has made it possible to coin the term Near Infrared Technology.

2.2. Foundation of Near Infrared Spectroscopy

The NIR region spans the wavelength range 780-2500 nm (Osborne, 1993; Blanco and Villarroya, 2002; Pasquini, 2003), in which absorption bands correspond mainly to overtones and combinations of fundamental vibrations (Figure 2.1). The vibration of molecules can be described using the harmonic oscillator model, by which the energy of the different, equally spaced levels can be calculated from:

$$E_{vib} = \left(\nu + \frac{1}{2} \right) \frac{h}{2\pi} \sqrt{\frac{k}{\mu}} \quad [2.1]$$

where ν is the vibrational quantum number, h the Planck constant, k the force constant and μ the reduced mass of the bonding atoms. Only those transitions between consecutive energy levels ($\Delta\nu = \pm 1$) that cause a change in dipole moment are possible,

$$\Delta E_{vib} = \Delta E_{rad} = h\nu \quad [2.2]$$

where ν is the fundamental vibrational frequency of the bond that yields an absorption band in the middle IR region.

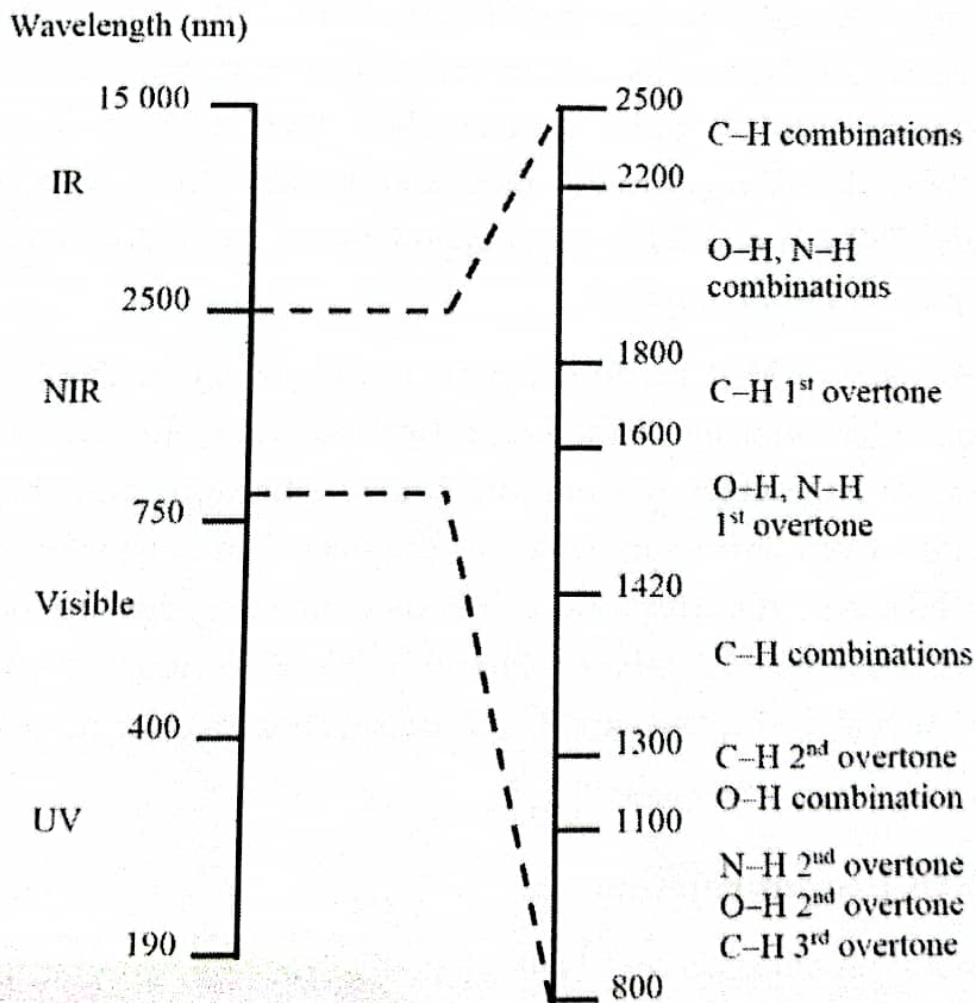


Figure 2.1. Principles of NIR absorption bands and their locations (adapted from Osborne, 1993).

However, the harmonic oscillator model cannot explain the behavior of actual molecules, as it does not take account of Coulombic repulsion between atoms or dissociation of bonds. As a result, the behavior of

molecules more closely resembles the model of an anharmonic oscillator, by which energy levels are not equally spaced. Thus, energy difference decreases with increasing ν :

$$\Delta E_{\text{vib}} = h\nu[1 - (2\nu + \Delta\nu; 1)y] \quad [2.3]$$

where y is the anharmonicity factor. The anharmonicity can result in transitions between vibrational energy states where $\Delta\nu = \pm 2, \pm 3, \dots$. These transitions between non-contiguous vibrational states yield absorption bands known as overtones (first and second overtone, respectively) at, approximately, multiples of the fundamental vibrational frequency. Also, they are much less likely than the fundamental transitions, so the bands are much weaker (the band for the first overtone is 10–100 times weaker than that for the fundamental frequency, depending on the particular bond). These bands appear between 780 nm and 2000 nm, depending on the overtone order and the bond nature and strength.

The intensity of NIR bands depends on the change in dipole moment and the anharmonicity of the bond. Because the hydrogen atom is the lightest, and therefore exhibits the largest vibrations and the greatest deviations from harmonic behavior, the main bands typically observed in the NIR region correspond to bonds containing this and other light atoms (namely C–H, N–H, O–H and S–H); by contrast, the bands for bonds such as C=O, C–C and C–Cl are much weaker or even absent.

2.3. NIR Instrumentation

Successful application of NIR depends on the correct choice of instrument. NIR spectroscopy instrumentation has evolved dramatically in response to the need for speed in analyses and flexibility in adapting to different sample states. Spectrophotometers used to record NIR spectra are essentially identical with those employed in other regions of the electromagnetic spectrum. But NIR equipment can incorporate a variety of devices (Figure 2.2) depending on the characteristics of the

sample and the particular analytical conditions and needs (such as speed, sample complexity and environmental conditions), so the technique is very flexible. NIR spectrophotometers can be of two types with respect to wavelength selection, namely discrete wavelength and whole spectrum. The former are simpler, as they irradiate samples with only a few wavelengths. As a result, they can be used in only applications with analytes absorbing in specific spectral zones. Whole-spectrum NIR instruments usually include a diffraction grating; although they may be of the Fourier transform (FT)-NIR type.

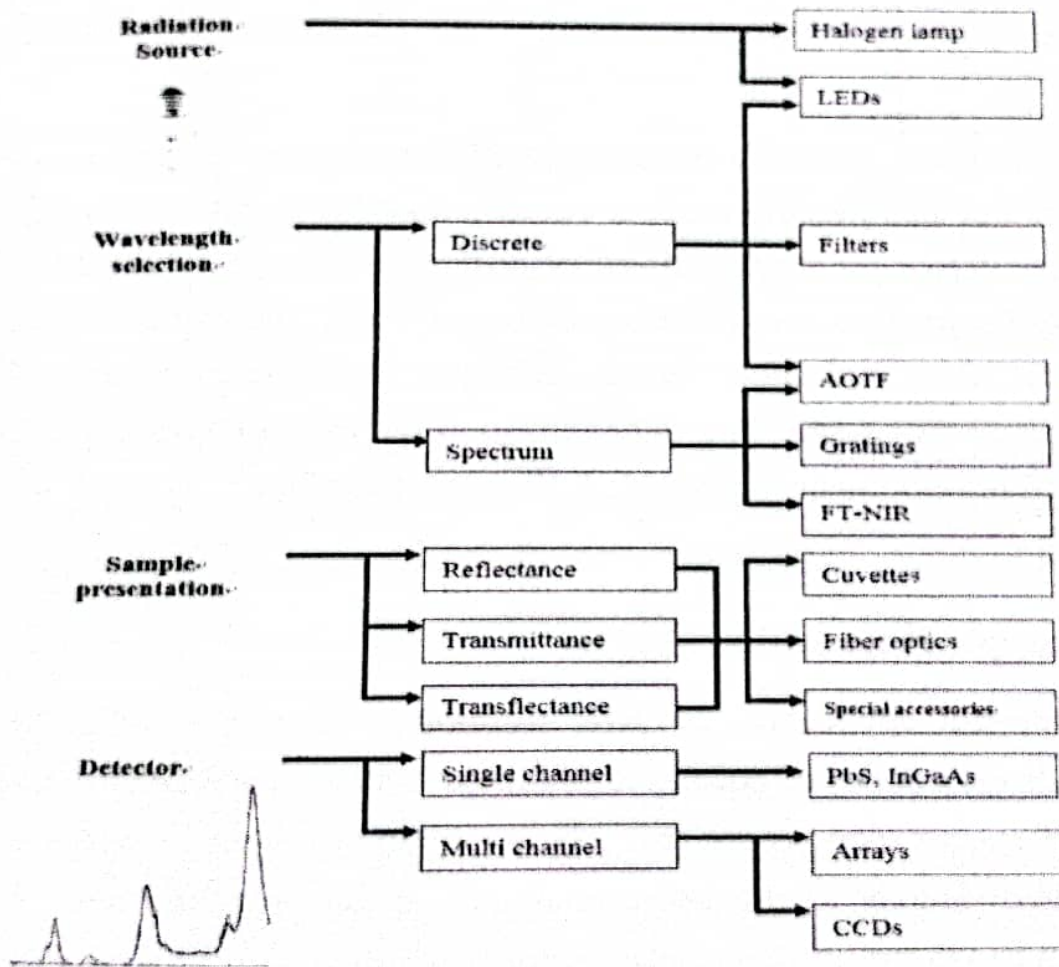


Figure 2.2. Principal features of NIR spectroscopy equipment (adapted from Blanco and Villaroya, 2002).

Detection in NIR spectroscopy uses devices comprising semiconductors (Si, PbS or InGaAs). A silicon detector (Si) covers the range 400–1100 nm; an indium gallium arsenide (InGaAs) covers the range 800–1700 nm and a lead sulfide the range 1100–2500 nm

(Osborne, 1993). In multi-channel detectors, several detection elements are arranged in rows (diode arrays) or planes [charged coupled devices (CCDs)] in order to record many wavelengths at once, so as to increase the speed at which spectral information can be acquired (Hanley *et al.*, 1996). This type of detector has given rise to NIR-imaging spectroscopy, in which spectra are recorded by using cameras that can determine composition at different points in space and record the shape and size of the object. Making measurements at different wavelengths provides a three-dimensional image that is a function of the spatial composition of the sample and the irradiation wavelength used.

Analysis is fast not only because the NIR technique records spectra quickly but also because there is virtually no need to pretreat samples. Another factor influencing speed of analysis is the ability to perform field measurements instead of having to collect samples for subsequent analysis in the laboratory. Some NIR spectrophotometers can make measurements on-site. The miniaturization of optical components has boosted development of portable NIR spectrophotometers.

2.4. Sample Presentation Modes

A successful application of NIR technology in the analytical field depends on a series of equally relevant factors. Most of the advantages of NIR spectroscopy come from the possibility of using intact samples presented directly to the instrument without any pre-treatment. This fact implies in promoting non-conventional interaction of the radiation with matter in order to extract the spectral information and generate many different measurement modes.

NIR spectrometry started as a unique technique when Karl Norris proposed that the spectral measurement could be obtained by analyzing the information content of that portion of radiation diffusely reflected by solid samples instead of the weaker signal of transmittance. Today, diffuse reflectance is one of the various possibilities for employing the

NIR spectral region. Figure 2.3 depicts the most common measurement modes employed by NIR spectroscopy.

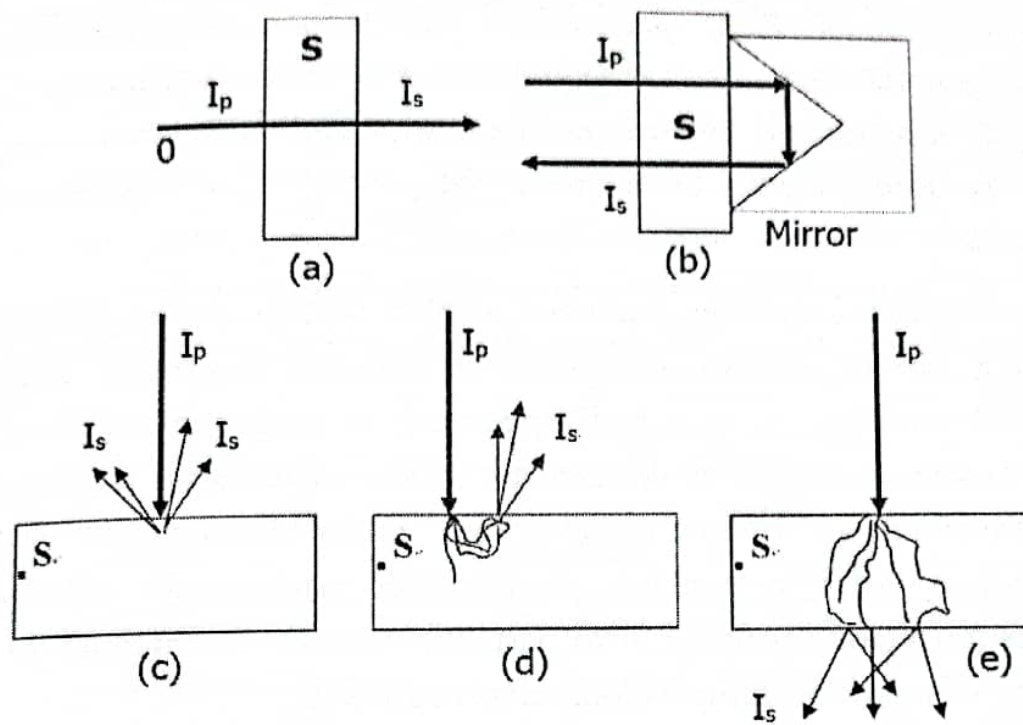


Figure 2.3. Modes of measurements employed in NIR spectroscopy. (a) transmittance; (b) transflectance; (c) diffuse reflectance; (d) interactance, and (e) transmittance through scattering medium (Adapted from Pasquini, 2003).

2.5. Developing a Calibration Model

NIR instruments determine protein and other components by measuring $\log(1/R)$ or $\log(1/T)$ values that must be related to the amount of the component as determined by some other method called a reference or standard method.

Obviously, the analytical information contained in the typically broad, extensively overlapped bands of NIR spectra is hardly selective and is influenced by a number of physical, chemical and structural variables. In addition, differences between samples may cause very slight spectral differences that are difficult to distinguish with the naked eye. For these

reasons, NIR spectroscopy requires chemometrics to extract as much relevant information as possible from the analytical data.

The two techniques (NIR spectroscopy and Chemometrics) are closely related, as NIR spectroscopy would never have reached its present stage of development without chemometrics and NIR spectroscopy results are frequently used to illustrate the power of new chemometric algorithms.

The analytical information contained in NIR spectra can be extracted by using various multivariate analysis techniques that relate several analytical variables (as in a NIR spectrum) to properties of interest (such as concentration) of the analyte(s). The multivariate techniques most frequently used allow samples with similar characteristics to be grouped, in order to establish classification methods for unknown samples (qualitative analysis) or to perform methods determining some property of unknown samples (quantitative analysis).

2.5.1. Multivariate Analysis Methods

The purpose of multivariate-analysis methods is to develop calibration models capable of accurately predicting the characteristics and properties of unknown samples. The process involves the steps described in Table 2.1.

Choosing the calibration samples

The most important factor in the step one is to ensure that the collected samples will take into account the variability of the samples in the future. For this reason, the collected samples should represent the range of prediction samples. However, if possible Gaussian distribution of samples with respect to composition should be avoided in the development of NIR calibration process. Calibrations developed with sample sets having Gaussian distribution of composition with respect

to a particular constituent may cause the results of future analyses to regress toward the mean (the "Dunne" effect) (Williams, 2001).

Determining the target parameter by using the reference method

The major limitation of NIR spectroscopy in food analysis is its dependence on less-precise reference method (Osborne, 1993). To be successfully developed a calibration model, it is important to ensure that we get a chemical data resulted from a precise and accepted reference method. The quality of chemical data measurement resulted from a reference method directly affects the quality of NIR calibration results.

Table 2.1. Steps in the multivariate model-development process.

No.	Steps	Purpose
1.	Choosing the calibration samples	To select a set of samples representative of the whole population
2.	Determining the target parameter by using the reference method	To determine the value of the measured property in accurate, precise manner.
3.	Recording the NIR spectra	To obtain physico-chemical information in a reproducible manner
4.	Subjecting spectra to appropriate treatments	To reduce unwanted contributions (such as shifts and scatter) to the spectra
5.	Developing the model	To establish the spectrum property relationship using multivariate methods
6.	Validating the model	To ensure that the model accurately predicts the property of interest in samples not subjecting to the calibration process

7.	Predicting unknown samples	To predict rapidly the property of interest in the new, unknown samples
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Subjecting spectra to appropriate treatments

After collecting spectral data and reference data with high accuracy, the next step is to construct the relationship between the two. However due to the complexity of the information contained in spectral data that is influenced by overlapping of the wavelength and the physical characteristic of the sample, it is highly required to treat the spectra before used. For example, the spectra of solid samples are influenced by the physical properties of the solid samples. This poses some problems in evaluating aspects of samples for which physical appearance is not important (such as identification of raw materials and determination of composition). In these situations, spectral pretreatment should be used to minimize those contributions incorporating irrelevant information into spectra in order to be able to develop more simple and robust models.

Some of the more frequent pretreatments for NIR spectra include: normalization (Griffiths, 1995); derivatives (usually first or second) (McClure, 1993); the multiplicative scatter correction (MSC) (Geladi *et al.*, 1985); the standard normal variate (SNV) and de-trending (DT) (Barnes *et al.*, 1989); or, a combination thereof.

Spectral data are often converted to derivatives to enhance the appearance and improve calibrations for constituents. The most common procedures are the use Savitzky-Golay equations and the gap procedures described by Norris and Williams (1984). Spectral data are also often smoothed to reduce the noise. The combination of derivative and smoothing can be applied to provide the optimum performance in predicting the constituent in samples.

Developing the calibration model

The next step is developing the calibration model, to correlate the spectral data and the reference one. For doing this task a number of multivariate analysis played an important role. The best known and most widely used is principal component analysis (PCA) (Wold *et al.*, 1987) and particularly partial least-squares (PLS) regression (Martens and Naes, 1991). These techniques rely on variable-reduction techniques that allow the dimensions of the original data to be reduced to a few uncorrelated variables containing only relevant information from the samples.

PCA searches for directions of maximum variability in sample groupings and uses them as new axes called "principal components" while PLS finds the directions of greatest variability by considering both spectral and target-property information, with the new axes called "PLS components" or "PLS factors". In this way, the relevant information for the system is contained in a reduced number of variables. The PCA data or PLS data thus obtained can be used as new variables, instead of the original data, in subsequent calculations.

Validating the model

"Validation" is the name of the process used to assess the performance of a calibration model. Although there is some disagreement over the exact meaning of term, the majority opinion at this time that it consists of ways to estimate the accuracy and robustness of any given calibration model at the time it is created.

The best way to determine these qualities is to have a set of samples, similar but separate from those used to create the calibration model, that also has known constituent compositions (normally from measurement made using the same reference laboratory procedure applied to the calibration samples themselves).

Cross-validation technique and t-test are frequently used for validating the calibration model. In t-test a validation process involves a new samples set that is not used in constructing the calibration model. If the sample set is small (up to 60), the calibration is best evaluated by using cross-validation. By this technique, all samples are used in development calibration model, and all are predicted; yet none of the samples is actually used in the calibration and validation process.

Predicting the unknown samples

The purpose of calibration model is to be used for routine control or rapid determination of the unknown samples in the future or in the process analytical technology. In this step, the best-developed calibration model is applied to the new samples to predict the constituents. This process has been replaced the task of many laborious wet chemical analysis. The relationship between calibration and prediction process was depicted in Figure 2.4. As pointed out in Figure 2.4, at first the best calibration model is selected and then this model will be used to predict the constituents in the routine analysis.

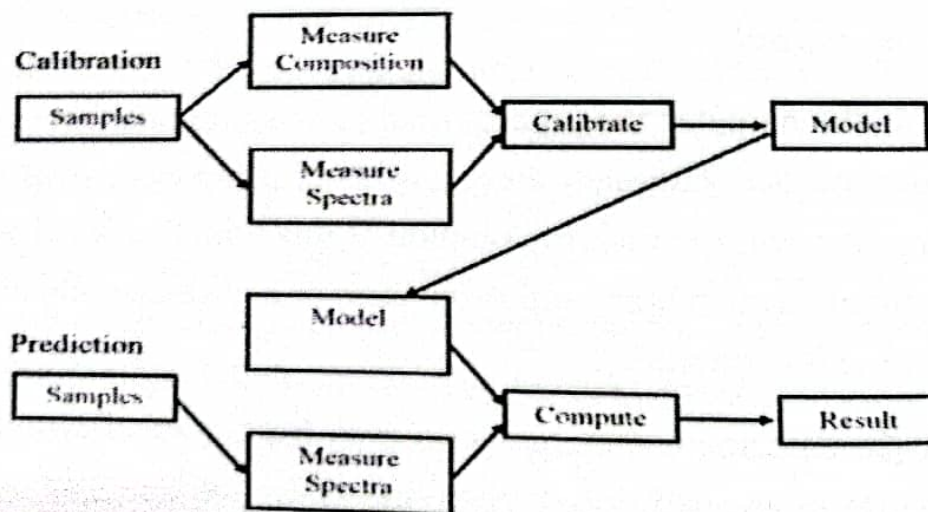


Figure 2.4. The relationship between calibration and prediction process in near infrared technology (adapted from Mark, 1993).

2.5.2. Evaluation of The Calibration Model

The efficiency of an NIR calibration is usually evaluated by means applied statistics. Several terms are needed for the correct interpretation of statistical analysis of the results of NIR testing. Unless all of these are correctly appraised, the operator may draw conclusions that are incorrect and can lead frustrating and often costly discrepancies. Some useful statistics are summarized in Table 2.2.

Different calibration models were calculated depending on the use of wavelength range, the pre-processing of the spectra and number of factor (or LV—latent variables) taken into consideration. In this study, the following two criteria were considered.

First, the quality of the calibration model was quantified by standard error of calibration (SEC), standard error of prediction (SEP) and multiple coefficient of determination (R^2) between the predicted and measured parameters. A good model should have a low SEC, a low SEP, a high correlation coefficient but also a small difference between SEC and SEP. A large difference indicates that too many latent variables are used in the model and noise is modeled (Gomez *et al.*, 2006).

Second, in general, a relative low number of factors are desirable to avoid the modeling of signal noise. The minimum of the plot of the root mean squared error of prediction (RMSEP) of the parameter of interest against the number of latent variables was used to determine the optimal number of latent variables. The correct number of regression factors for the PLS and PCR models was determined by minimum of root mean square error of cross validation. More variables result in an “over-fitted” model, while fewer produced an “under-fitted” model.

Table 2.2. Statistical terms used for evaluation of calibration model.

Terms	Equations
R^2	$R^2 = \left[\frac{\sum(x \times y) - [(\sum x \times \sum y) / N]}{\left\{ \left[\sum x^2 - [(\sum x)^2 / N] \right] \times \left[\sum y^2 - [(\sum y)^2 / N] \right] \right\}^{1/2}} \right]^2$
SEC	$SEC = \left\{ \sum(x - y)^2 - \left[\frac{[\sum(x - y)]^2}{N} \right] / N - 1 \right\}^{1/2}$
SEP	$SEP = \left\{ \sum(x - y)^2 - \left[\frac{[\sum(x - y)]^2}{N} \right] / N - 1 \right\}^{1/2}$
Bias	$bias = \sum(x - y) / N$
RPD	$RPD = \frac{SD_{calib}}{SEP}$

x : Reference values

y : NIR predicted values

N : Number of samples

R^2 : The multiple coefficient of determination between reference and NIR predicted values

SEC: The standard deviation (SD) of differences between reference and NIR predicted values in the calibration sample set

SEP: The standard deviation (SD) of differences between reference and NIR predicted values in the validation sample set

Bias: The average difference between reference and NIR predicted values

RPD: The ratio of SEP to standard deviation (SD) of the validation sample set

References

- Barnes R.J., Dhanoa M.S., Lister S.J. 1989. Standard normal variate transformation and de-trending of near infrared diffuse reflectance spectra. *Applied Spectroscopy*. 43: 772-777.
- Blanco, M., Villarroya, I. 2002. NIR spectroscopy: a rapid-response analytical tool. *Trends in Analytical Chemistry*. 21(4): 240-250.
- Geladi P, MacDougall D, Martens H. 1985. Linearization and scatter-correction for near infrared reflectance spectra of meat. *Applied Spectroscopy*. 39: 491-500.
- Gómez, A.H., He, Y., Pereira, A.G. 2006. Nondestructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis/NIR-spectroscopy techniques. *J. Food Engineering*. 77: 313-319.
- Griffiths, P.R. 1995. Letter: Math pretreatment of NIR reflectance data: $\log(1/R)$ vs $F(R)$. *J. Near Infrared*. 3(1): 60-62.
- Hanley, Q.S., Earle, C.W., Pennebaker, F.M., Madden, S.P., Denton, M.B. 1996. Charge-transfer devices in analytical instrumentation. *Anal. Chem*. 68(21): 661A-667A.
- Mark, H. 1993. Quantitative spectroscopic calibration. In *Encyclopedia of Analytical Chemistry*. John Wiley and Sons Ltd, Chichester. ISBN: 0471976709.
- Martens, H., Naes, T. 1991. *Multivariate calibration*. John Wiley, New York, NY. 419 pp.
- McClure, W. F. 1993. More on derivatives: Part 1. Segments, gaps and ghosts. *NIR news*. 4(6): 12.
- Norris, K.H., Williams, P.C. 1984. Optimization of mathematical treatments of raw near infrared signal in the measurement of protein in hard red spring wheat. I. Influence of particle size. *Cereal Chem*. 61: 158-165.

Osborne, B.G. 1993. Near infrared spectroscopy in food analysis. In Encyclopedia of Analytical Chemistry. John Wiley and Sons Ltd, Chichester. ISBN: 0471976709.

Pasquini, C. 2003. Near infrared spectroscopy: fundamentals, practical aspects and analytical applications. J. Braz. Chem. Soc. 14(2): 198-219.

Williams, P.C. 2001. Implementation of near infrared technology. In "Near-infrared technology in the agriculture and food industries 2nd edition" (ed. by Williams, P. and Norris, K.). Am. Soc. of Cereal Chemists Inc., St. Paul Minn., p 152-153.

Wold, S., Esbensen, K., Geladi, P. 1987. Principal component analysis Chemom. Int. Lab. Syst. 2(1-3): 37-52.

The Application of Near Infrared Spectroscopy and Chemometrics

for Monitoring Water Stress in Tomato Plants

Irrigation control in agriculture requires the accuracy of the timing of irrigation and the amount of water to be added. Especially in a protected cultivation, in order to increase the sugar content of tomato fruits, the development of a technique to reduce water supply and to impose proper water stress is needed. Yet, in most of the cases, the water stress management is controlled by farmer's experience. To optimize the benefit of the water stress, it is highly required to have an accurate, easy to use and nondestructive method of measuring plant water stress. Using such a method, a more accurate irrigation control system that used plant-response based feedback would be possible. Leaf water potential (LWP) has been regarded as a good indicator of plant water stress. To realize the optimization of water stress, it is effective to monitor the LWP values of the tomato plant.

This book reported the use of near infrared technology as a nondestructive method to measure the LWP in tomato plants. This measurement open a possible application to control the LWP in tomato plants. Finally we reported the water stress management in tomato plants to produce tomato fruits with high sugar content.



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