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ANALYSIS OF EDIBLE OILS BY FOURIER TRANSFORM NEAR-INFRARED SPECTROSCOPY

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Suggested Short Title: OIL ANALYSIS BY FT-NIR SPECTROSCOPY

Dedicated to my parents and my husband

ABSTRACT

Fourier transform near-infrared (FT-NIR) spectroscopy was investigated as a means of quantitative analysis of edible fats and oils. Initially, a method of simultaneously determining the *cis* and *trans* content, iodine value and saponification number of neat fats and oils using a heated transmission flow cell was developed. Two partial least squares (PLS) calibrations were devised, a process-specific calibration based on hydrogenated soybean oil and a more generalized calibration based on many oil types, the latter able to analyze oils from a variety of sources accurately and reproducibly. Methodology for the quantitative determination of the peroxide value (PV) of edible oils using a novel glass-vial sample handling system was subsequently developed, based on the stoichiometric reaction of triphenylphosphine with hydroperoxides to form triphenylphosphine oxide. The PV calibration was derived using PLS regression, and the results of a validation study demonstrated that PV could be quantitated accurately if a normalization routine was used to compensate for the inherent dimensional variability of the vials. The vial sample handling system was then used in the development of PLS IV calibrations for the process analysis of commercial oil samples, and these samples were also used to evaluate a global IV calibration devised by Bomem Inc. The discriminant features available through PLS were shown to enhance the accuracy of the IV predictions by facilitating the selection of the most appropriate calibrations based on the spectral characteristics of closely related oils. The predictions obtained using the global IV calibration provided clear evidence that a generalized calibration based on a large and varied selection of oils could provide a means of IV determination by FT-NIR spectroscopy. Subsequently, a generalized FT-NIR *trans* calibration was developed and shown to yield *trans* values that were in good agreement with those obtained by the AOCS mid-FTIR single-bounce horizontal attenuated total reflectance method, having an accuracy and reproducibility of ±1.1 and ±0.5% *trans*, respectively. The results of the research demonstrate that FT-NIR spectroscopy coupled with the glass-vial sample handling system is a viable alternative to mid-FTIR spectroscopy for the analysis of edible oils and could be suitable as an industrial at- or on-line quality control tool.

RÉSUMÉ

L'utilisation de la spectroscopie près de l'infrarouge à transformation de Fourier (FT-NIR) en tant que véhicule d'analyse quantitative des gras ainsi que des huiles comestibles non traitées a été étudiée. Dans un premier temps une méthode ayant pour but de procéder à la détermination simultanée du contenu *cis* et *trans*, de l'indice d'iode (IV) ainsi que du nombre de saponification a été développée en ayant recours à une cellule de transmission à circulation chauffante . Deux calibrages basés sur la moindre partielle des carrés (PLS) ont été développés. Dans le premier cas un calibrage plus spécifique fut établi basé sur l'utilisation d'huile de soya hydrogénée. Un calibrage plus universel fut ensuite effectué reposant sur l'utilisation de différents types d'huiles de diverses provenances tout en maintenant un niveau adéquat de précision. Une méthodologie visant à permettre une détermination quantitative de la valeur de peroxyde (PV) des huiles comestibles fut effectuée en ayant recours à un nouveau dispositif permettant la manipulation de contenants d'échantillons de mesures différentes se basant sur la réaction stœchiométrique du triphenylphosphine avec les hydropéroxydes et résultant en la formation d'oxyde de triphenylphosphine. Le calibrage PV fut obtenu en utilisant une régression basée sur la moindre partielle des carrés (PLS) et la validation des résultats a permis de démontrer qu'il était possible de quantifier la valeur de peroxyde (PV) à l'aide d'un facteur de correction normalisé tenant compte des variations dans les dimensions des contenants. Ce nouveau système fut par la suite utilisé afin de procéder à l'analyse en cours de production d'échantillons d'huiles commerciales. Il servit aussi de référence afin de procéder à l'évaluation de la méthode de calibrage « Global IV » conçue par Bomem Inc. L'utilisation de PLS a permis d'augmenter la précision des prédictions facilitant la sélection des calibrages les plus appropriés basés sur les caractéristiques spectrales d'huiles et ce, même dans les cas où elles sont extrêmement similaires. Les prédictions obtenues à l'aide du « Global IV » ont démontré clairement qu'une méthodologie de calibrage généralisée basée sur une grande sélection/variété d'huiles peut fournir un moyen de détermination fiable de l'indice d'iode (IV) à l'aide de FT-NIR. Cette étude servit de base à l'élaboration d'une méthode FT-NIR capable de prédire des valeurs *trans* dans une grande variété d'huiles en concordance avec les méthodes mid-FTIR de

réflectance totale atténuée n'ayant qu'un point de réflexion (SB-HATR) de l'AOCS avec un niveau de précision de ± 1.1 et reproductible à ± 0.05%. Le travail effectué prouve donc que l'utilisation de la spectroscopie FT-NIR tenant compte des variations dans les mesures des contenants d'échantillons se compare avantageusement à la spectroscopie mid-FTIR en ce qui a trait à l'analyse des huiles comestibles et se démarque clairement comme une alternative en tant qu'outil d'assurance qualité à l'interne ou même directement sur la ligne de production.

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CONTRIBUTIONS OF AUTHORS

Four published manuscripts constitute the bulk of the research work carried out for this thesis. The author, Hui Li, was responsible for the concepts, design of experiments, experimental work and manuscript preparation. Drs. van de Voort and Ismail are thesis supervisor and thesis advisor, respectively, and had direct advisory input into the work as it progressed. Dr. J. Sedman was a chemometric specialist and programming advisor, while Drs. Cox, Simard and Buijs are researchers at ABB Bomem Inc., industrial partners in this research, who contributed instrumental resources and expertise to the work carried out.

List of the publications reproduced in the thesis:

Hui Li, F. R. van de Voort, A. A. Ismail, and R. Cox, *Trans* Determination Of Edible Oils By Fourier Transform Near-Infrared (FT-NIR) Spectroscopy, *J. Am. Oil Chem. Soc.* (*in press*)

Hui Li, F. R. van de Voort, A. A. Ismail, and R. Cox, Determination of Peroxide Value by Fourier Transform Near-Infrared (FT-NIR) Spectroscopy, *J. Am. Oil Chem. Soc.* 77:137-142 (2000)

Hui Li, F. R. van de Voort, , A. A. Ismail, J. Sedman, R. Cox, C. Simard and H. Buijs, Discrimination of Edible Oil Products and Quantitative Determination of Their Iodine Value by FT-NIR Spectroscopy, *J. Am. Oil Chem. Soc.*77:29-36 (2000)

Hui Li, F. R. van de Voort, J. Sedman, and A. A. Ismail, Rapid Determination of *cis* and *trans* Content, Iodine Value and Saponification Number of Edible Oils by Fourier Transform Near-Infrared (FT-NIR) Spectroscopy, *J. Am. Oil Chem. Soc.* 76:491-497 (1999)

The following paper is related to this work, but has not been included in the thesis.

Cox, R., J. Labrassier, E. Michiels, and H. Buijs, **Hui Li**, F. R. van de Voort, A. A. Ismail, and J. Sedman, Determination of Iodine Value by Fourier Transform Near-Infrared Spectroscopy – A Collaborative Study, *J. Am. Oil Chem. Soc.* (submitted)

CONTRIBUTIONS TO KNOWLEDGE

(1) Demonstrated that the rapid and simultaneous determination of *cis* and *trans* content, iodine value and saponification number of edible fats and oils by Fourier transform near-infrared spectroscopy using a transmission flow cell was possible.

The method developed demonstrates that an FT-NIR spectrometer can be calibrated for the prediction of these four parameters using mid-FTIR data and that the FT-NIR method provides a rapid alternative to the commonly used chemical and physical methods presently employed in the industry, an analysis only taking two minutes per sample.

(2) Refined a method for the determination of peroxide value (PV) of edible fats and oils by Fourier transform near-infrared spectroscopy to allow low levels of PV to be accurately determined using a disposable glass-vial sample handling accessory.

The method developed allows the accurate analysis of PV between 0 and 10, a range of interest to the edible oil industry. The method developed uses convenient disposable glass vials for sample handling, and PV is determined by spectroscopically measuring the conversion of triphenylphosphine to triphenylphosphine oxide when reacted with hydroperoxides.

(3) Developed oil-specific FT-NIR calibrations for the determination of IV and implemented discrimination procedures to select appropriate calibrations and detect outliers as well as validating a "global" IV calibration developed by Bomem.

Discriminate analysis was successfully used to select calibrations based on IV, spectral characteristics and/or factor scores and the performance of the oil-specific calibrations and the global calibration were validated using processor samples.

(4) Developed a generalized FT-NIR *trans* determination method for edible fats and oils

The FT-NIR *trans* calibration was developed from a wide selection of edible oils and validated using processor samples. Oil-specific calibrations were also developed and utilized to select the most appropriate calibration based on the spectral characteristics of the oil.

(5) Have demonstrated that the disposable glass-vial sample handling system can be used for quantitative FT-NIR analysis.

The need for a constant pathlength is a common assumption in spectroscopic analyses. It has been demonstrated that a degree of vial variability can be tolerated and accounted for through the use of a spectral normalization routine, facilitatiing the

use of cheap, simple to use glass vials for FT-NIR analysis.

(6) Illustrated the utility of PLS as a discriminant tool in the analysis of edible fats and oils by FT-NIR spectroscopy

In quality control situations analysis of many oil types may take place, involving multiple calibrations. Discriminate analysis can be used to select the correct calibration based on selected characteristics and avoid applying the wrong calibration to the wrong sample or detect anomalous samples.

(7) Have submitted the FT-NIR IV method for consideration as official method to the American Oil Chemists Society (AOCS).

Based on this research, a collaborative study was carried out and the results submitted to the AOCS for consideration of FT-NIR IV determination as an official method of analysis, which is presently pending approval. This is the first FT-NIR quantitative instrumental method considered in the fats and oils sector.

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LIST OF ABBREVIATIONS AND SYMBOLS

μm	Micrometer
AOM	Active oxygen method
AgBr	Silver bromide
AgCl	Silver chloride
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
ATR	Attenuated total reflectance
AV	Anisidine value
BaF2	Barium fluoride
CaF2	Calcium fluoride
CLS	Classic least squares
CO_2	Carbon dioxide
CRD	Collaborative Research and Development
CS_2	Carbon disulfide
CsI	Cesium iodide
DTGS	Deuterated triglycine sulfate
%DV	Percent Daily Value
EOA	Edible oil analysis
FDA	Food and Drug Administration
FFA(s)	Free fatty acid(s)
FTIR	Fourier transform infrared spectroscopy
FT-NIR	Fourier transform near infrared spectroscopy
GC	Gas chromatography
Ge	Germanium
HATR	Horizontal attenuated total reflectance
HPLC	High pressure liquid chromatography
ILS	Inverse least squares
InGAs	Indium gallium arsenide
IR	Infrared
IRE	Internal reflection element

IUPAC	International Union of Pure and Applied Chemistry
IV	Iodine value
KBr	Potassium bromide
KCl	Potassium chloride
MCT	Mercury cadmium telluride
MDa	Mean difference of accuracy
MD_r	Mean difference of reproducibility
NaCl	Sodium chloride
NIR	Near infrared spectroscopy
NMR	Nuclear magnetic resonance
NSERC	Natural Sciences and Engineering Research Council
PCA	Principal component analysis
PCR	Principal component regression
PLS	Partial least squares
PRESS	Predicted residual error sum of squares
PV	Peroxide value
RMSE	Root mean square error
SB-HATR	Single-bounce horizontal attenuated total reflectance
SD	Standard deviation
SDDa	Standard deviation of difference of accuracy
SDDr	Standard deviation of difference of reproducibility
SE	Standard error
SEP	Standard error of prediction
SFC	Solid fat content
SFI	Solid fat index
SiO_2	Silicon oxide
SN	Saponification number
S/N	Signal-to-noise
TBA	Thiobarbituric acid
TBHP	Tertiary butyl hydroperoxide
TFA	*trans* fatty acid

TPP	**Triphenylphosphine**
TPPO	**Triphenylphosphine oxide**
ZnS	**Zinc sulfide**
ZnSe	**Zinc selenide**

CHAPTER 1 GENERAL INTRODUCTION

Fats and oils are recognized as essential nutrients in the human diet, being consumed in a variety of forms. In the fats and oils industry, routine analyses are generally carried out using standardized chemical and physical methods sanctioned by the American Oil Chemists Society (AOCS) and/or the Association of Official Analytical Chemists (AOAC). However, since these methods tend to be tedious, time-consuming, and expensive and often require hazardous solvents and reagents, there is a growing demand within the industry to replace these methods with automated instrumental methods.

Infrared (IR) spectroscopy is a powerful technique for qualitative analysis as the IR spectrum contains detailed and unique information about the chemical composition and molecular structure of a substance. Although IR spectroscopy has been used extensively for the qualitative analysis of fats and oils, quantitative analysis has largely been restricted to the determination of isolated *trans* isomers. This has been due to the limitations of traditional dispersive spectrometers, problems associated with sample handling and the lack of the computational capabilities required for quantitative analysis of multicomponent systems. This situation changed with the development of Fourier transform infrared (FTIR) spectroscopy, paralleled with advances in data manipulation techniques and chemometrics. Reductions in the capital cost of FTIR instrumentation in the late 1980s led to consideration of FTIR spectroscopy as a quantitative quality control and process monitoring tool in the fats and oils industry.

A variety of indicator parameters are used to assess the quality of edible fats and oils, including peroxide value (PV), anisidine value (AV), saponification number (SN), iodine value (IV), free fatty acid (FFA) content, and solid fat content (SFC), to name but a few. The information provided by an infrared spectrum of an oil is substantial and can often be related to such parameters. For example, the OH stretching region of the IR spectrum can indicate the presence of moisture or hydroperoxides, the latter commonly measured by the chemical PV test, while the intensities of the bands in the CH region and of the ester linkage absorption are related to the average molecular weight of an oil, commonly

evaluated by SN determinations. The COOH absorption is indicative of lipolysis or free fatty acid (FFA) content of an oil, while the presence of spectral features related to aldehydes and ketones as well as conjugated dienes is indicative of the accumulation of secondary oxidation products commonly measured by the thiobarbituric acid (TBA) test or the AV test. The isolated *trans* band provides a direct measure of isolated *trans* isomers while the combination of CH *cis* and CH *trans* absorptions provides a measure of total unsaturation or IV. The complete spectrum of a refined, melted fat characterizes its overall triglyceride composition, which in turn can be related to its SFC, which could also provide information on the relative amounts of saturated, monounsaturated, and polyunsaturated fatty acids in a fat or oil. Linking the information available from an IR spectrum to quality or chemical parameters of an oil in a quantitative manner would be of great benefit to the edible oils industry for quality control purposes.

In 1990, the McGill IR Group was formed to advance the application of FTIR spectroscopy to food and edible oil analysis based on extending the potential utility of this analytical technique to the industry. Extensive research has been carried out over the past 10 years and a wide variety of mid-IR methods have been developed, demonstrating that mid-FTIR spectroscopy can be utilized as a rapid analytical quality control tool. However, the possibilities of extending this methodology to address the needs of the industry for at- and on-line analysis are restricted by inherent limitations of mid-IR spectroscopy.

A key problem related to in-plant QC analysis by mid-FTIR spectroscopy is that most of the available instrumentation is not designed for operation in an industrial processing environment. Vibration and variations in temperature and humidity strongly affect instrument performance, limiting most FTIR instruments to the laboratory. In addition few reasonably priced instruments are sufficiently ruggedized to withstand the relatively harsh conditions in a processing plant, where, for example, equipment is often hosed down. The ideal solution to these problems would be to locate the spectrometer in a controlled environment and link it via fiber optics to one or more sampling locations, but this alternative is not highly practicable at the present time owing to the expense and low

optical throughput of mid-IR-transmitting fiber optics. The possibilities for at-line or on-line analysis are also severely restricted by the sample handling limitations inherent to mid-IR spectroscopy. These limitations include the requirement for short-pathlength cells (typically 0.025-0.1 mm for oil analysis), owing to the low energy output of mid-IR radiation sources, and the limited selection of mid-IR transmitting cell window materials, the commonly used materials being alkali halide salt crystals, which are water soluble and easily fogged in a humid atmosphere.

On the other hand, to a large degree, many of these limitations can be overcome by switching to the near-IR (NIR) region. Summarizing, NIR instruments have greater detector sensitivity, substantially more energy is available from the source, and absorptivities are low in the NIR region, making it possible to use longer pathlengths (e.g., 1-10 cm). In addition, quartz and even silica glass are suitable as window materials, allowing the use of simple, disposable glass vials. The longer pathlengths facilitate the use of flow cells for on-line applications, and the availability of low-cost fiber optics allows for remote analysis if desired. Furthermore, NIR instruments have been around for many years and have found a wide range of applications in food analysis, and NIR instruments are well-recognized in-plant quality control tools. However, the traditional dispersive and filter instruments are hampered by the problem of calibration drift due to wavelength instability over time. This problem is overcome with the advent of commercial FT-NIR instruments, a laser reference in FT-NIR instruments providing exact interferometer positioning, which in turn provides wavelength stability and reproducibility. As a consequence, wavelength drifts over time and the resulting problem of calibration drift are eliminated.

It is the hypothesis of this thesis that it should be possible to convert many of the mid-IR methods developed for the analysis of fats and oils to FT-NIR methods. In order to take full advantage of the sample-handling flexibility of NIR spectroscopy, the research described in this thesis is concerned not only with FT-NIR methodology development but also with the evaluation of a new sample handling system that employs disposable glass vials. Chapter 3 focuses on the conversion of a generalized mid-IR analytical package for

the simultaneous determination of *cis* and *trans* content, IV and SN to a FT-NIR method using a conventional constant-pathlength transmission cell. In Chapter 4, an evaluation of the vial sample handling system is made in relation to the development of a FT-NIR method for the determination of peroxide value (PV) and comparison of its performance to that of a well-characterized mid-IR PV method. Chapter 5, which focuses specifically on IV analysis using the vial accessory, describes the development of calibrations for specific commercial oil formulations, assesses a global IV calibration, and explores the discriminate analysis features of PLS. Chapter 6 focuses on devising a generalized FT-NIR *trans* calibration and the implementation of discriminate analysis. Overall, the research presented in these four chapters serves to examine the potential utility of FT-NIR spectroscopy is an alternative to mid-FTIR spectroscopy as an analytical tool for the analysis of fats and oils, and the conclusions drawn from the results of this research are presented in the final chapter.

CHAPTER 2 LITERATURE REVIEW

IR spectroscopy has a long and illustrious history and has been one of the main tools available to chemists to obtain detailed information about the structure of molecules. Given its broad scope and wide use, out of necessity this literature review can only focus on aspects relevant to the research carried out in this thesis. This literature review will highlight the basic principles of IR spectroscopy, relevant instrumentation, applicable sample handling techniques, chemometric basics, as well as previous research relevant to the work carried out.

2.2 Principles of Infrared Spectroscopy

Electromagnetic radiation, of which the IR is a part, may be considered as a simple undulating electro-magnetic harmonic wave, the undulating forces being interconnected electric and magnetic fields which interact with matter to give rise to IR absorptions (1). Table 2.1 tabulates the regions of the electromagnetic spectrum in terms of wavenumber, frequency and energy relative to the position of IR (2).

The IR region of the electromagnetic spectrum extends from 10 to 14300 cm^{-1}, and is conveniently divided, both instrumentally and functionally, into near-, mid- and far- IR (Table 2.2). When IR frequencies pass through a sample, some of the frequencies are absorbed, while others are transmitted through the sample without being absorbed and an IR spectrum is a plot of absorbance or transmittance against frequency or wavelength.

Molecular absorption of electromagnetic radiation over the IR region of the spectrum promotes molecular transitions between rotational and vibrational energy levels of the ground (lowest) electronic energy state (3). In the mid-IR and NIR regions, only vibrational modes are of concern and it is important to note that only the vibrations resulting from a molecular dipole moment change give rise to IR absorption bands.

Table 2.1. Regions of the electromagnetic spectrum

Name	wavelength in vacuo, λ_0	Wavenumber in vacuo, ν	Frequency ν	photon energy $h\nu$	molar energy $N_A h\nu$
γ-rays	10pm	10^9 cm^{-1}	30.0Ehz	19.9x 10^{-15} J	12.0 GJ/mol
X-rays	10nm	10^6 cm^{-1}	30.0PHz	19.9x 10^{-18} J	12.0 MJ/mol
Vacuum UV	200nm	50.0x 10^3 cm^{-1}	1.50PHz	993x 10^{-21} J	598 kJ/mol
Near UV	380nm	26.3x 10^3 cm^{-1}	789THz	523x 10^{-21} J	315 kJ/mol
Visible	780nm	12.8x 10^3 cm^{-1}	384THz	255x 10^{-21} J	153 kJ/mol
Near-IR	2.5μm	4.0x 10^3 cm^{-1}	120THz	79.5x 10^{-21} J	47.9 kJ/mol
Mid-IR	50μm	200 cm^{-1}	6.00THz	3.98x 10^{-21} J	2.40 kJ/mol
Far-IR	1 mm	10 cm^{-1}	300GHz	199x 10^{-24} J	120 J/mol
Microwaves	100mm	0.1 cm^{-1}	3.00GHz	1.99x 10^{-24} J	1.20 J/mol

IR stands for infrared and UV for ultraviolet. *H* stands for the Planck constant and N_A for the Avogadro constant

Table 2.2. Divisions of the infrared region (1)

Region	Characteristic transitions	Wavelength range (nm)	Wavenumber range (cm^{-1})
Near infrared (NIR)	Overtones Combinations	700-2500	14300-4000
Middle infrared (MIR)	Fundamental vibrations	2500-5x10^4	4000-200
Far infrared	Rotations	5x10^4-10^6	200-10

A non-linear molecule of *n* atoms has 3*n* degrees of freedom, which are distributed as 3 rotational, 3 transitional, and 3*n*-6 vibrational motions, each with a characteristic fundamental band frequency. These vibrational motions can be described in terms of bond stretching and various types of bending vibrations as presented diagrammatically in Figure 2.1 for a tri-atomic molecule or group AX_2 (4). In the case of a linear molecule, there are only two rotational degrees of freedom, since rotation about the bond axis is not possible and therefore, a linear AX_2 molecule has 3*n*-5 normal vibrational motions.

In a simple A-B diatomic molecular model the only vibration that can occur is a periodic stretching along the A-B bond. This stretching vibration resembles the oscillations of two bodies connected by a spring. Thus, to a first approximation, the model of a harmonic oscillator can be used to describe this vibration, and the restoring force (F) on the bond is then given by Hooke's law:

$$F = -kx \qquad [2.1]$$

where k is the force constant of the bond, and x is the displacement. Using this approximation, the vibrational frequency ν is then given by:

$$\nu = (1/2\pi c)(k/\mu)^{1/2} \qquad [2.2]$$

where c is the velocity of light, and μ the reduced mass of the system, as defined by the following equation:

$$\mu = m_A . m_B / (m_A + m_B) \qquad [2.3]$$

where m_A and m_B are the individual masses of A and B.

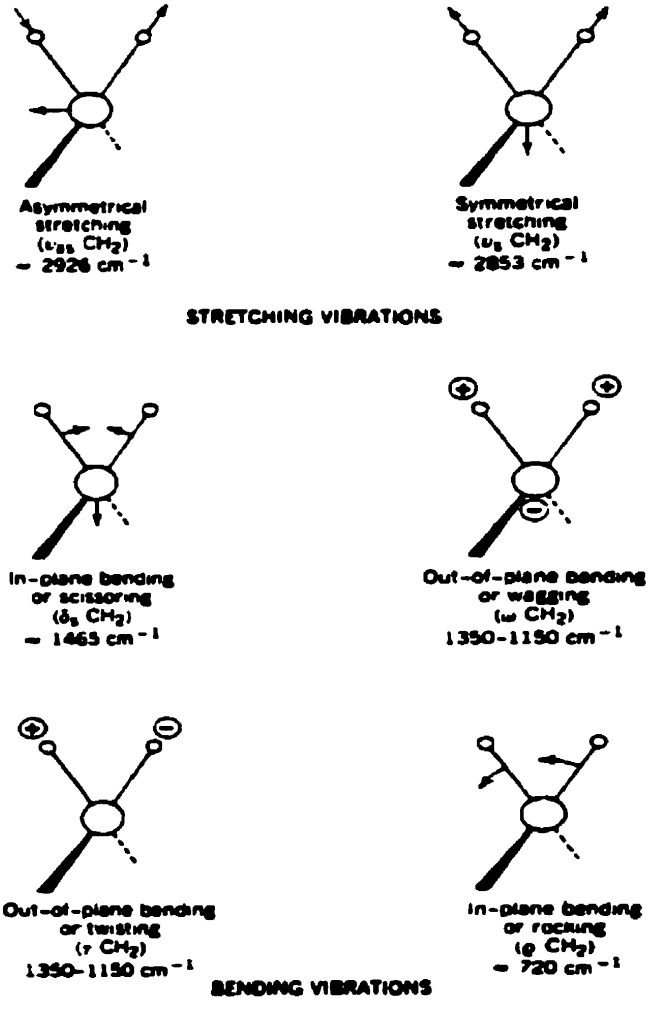

Figure 2.1. Vibrational modes for an AX_2 group. (+ and − indicate movement perpendicular to the plane of the page)

In reality, molecular vibrations are somewhat more complicated than a simple harmonic oscillator model and involve quantum mechanics. While the Hooke's law would indicate a continuum of vibrational states, the molecular vibrational energy levels are in fact quantized:

$$E = (n+1/2)h\nu \qquad [2.4]$$

where h is Planck's constant, n is the vibrational quantum number, and ν is the fundamental vibrational frequency. Accordingly, a molecule will absorb IR radiation of a particular frequency only when this frequency matches the frequency of one of the molecule's fundamental vibrational modes.

Molecular absorption of energy in the mid-IR region of electromagnetic spectrum (4000-200 cm^{-1}) is related to the fundamental vibrational modes, which correspond to transitions from the ground-state vibrational level (n=0) to the first excited vibrational level (n=1). Equation [2.2] is the basis for group frequency determinations in mid-IR region, the force constant k, and the relative masses of the bonded atoms constituting the two most important factors determining frequency. There are however a host of other factors, both internal and external with respect to the molecule, which dictate the actual absorption frequency observed. These include electrical effects, steric effects, the nature, size and electronegativity of neighboring atoms, phase changes and hydrogen bonding, all of which individually, or in concert, may cause shifts in the expected frequency.

Even though there are a variety of secondary factors that can affect the frequency observed, defined functional groups do exhibit fairly characteristic absorption frequencies that allow their presence in a compound to be established from its IR spectrum. Thus the pattern of absorbed wavelengths for a molecule, its mid-IR spectrum, can be regarded as a molecular "fingerprint", making mid-IR spectroscopy a very powerful qualitative analytical tool that can be employed for the identification of chemical compounds and the elucidation of molecular structure. Mid-IR spectroscopy can also be used for quantitative

analysis because the amount of IR energy absorbed by a compound is proportional to its concentration in the sample (Beer's Law).

As indicated by equation [2.4] in the harmonic oscillator approximation, quantum-mechanical treatment predicts equally spaced vibrational energy levels. The equation also suggests that only transitions from the ground-state vibrational level (n=0) to the first excited vibrational level (n=1) will be observed (5) at room temperature. However, weak overtone bands, corresponding to transitions to higher vibrational levels, are always observed experimentally. Due to the anharmonic nature of molecular vibrations, the overtone frequencies fall somewhat short of being integral multiples of the fundamental vibrational frequency. Anharmonicity also gives rise to combination bands, which represent the simultaneous excitation of two fundamental vibrational modes. Because overtone and combination bands are forbidden bands, they are approximately an order of magnitude weaker than fundamental modes.

NIR spectroscopy, in contrast to mid-IR spectroscopy, is concerned with the study of overtones and combination bands, which are predominantly found in the NIR region of the spectrum (12,800-4000cm^{-1}). Generally speaking, there are four partially overlapping band regions in the NIR: combination bands, 1950-2500 nm (5100-4000 cm $^{-1}$); first overtone bands, 1500-2000 nm (6667-5000 cm $^{-1}$); second overtone bands, 1050-1650 nm (9524-6061 cm $^{-1}$); and third overtone bands, 700-1150 nm (142857-8695 cm $^{-1}$). Bonds involving hydrogen, the lightest of the atoms, vibrate with larger amplitude when undergoing stretching and as a consequence its motions deviate most appreciably from harmonic vibrations and result in overtones. Consequently, almost all the absorption bands observed in the NIR portion of the spectrum arise from overtones of hydrogen stretching vibrations involving AH_y functional groups or combinations involving stretching and bending modes of vibration of such groups. The bands in NIR region overlap extensively and result in extremely diffuse and complex spectra that are poorly resolved. Thus, a NIR spectrum does not provide any of the detailed spectral information discernable by inspection from a mid-IR spectrum. However, with new sophisticated and

advanced chemometric tools available substantial qualitative and quantitative information can be extracted.

2.3 Instrumentation

Based on how IR radiation is resolved into its component wavelengths, IR spectrometers can be divided into two general categories: dispersive and Fourier transform IR spectrometers. In the 1940s, the first commercial dispersive scanning IR spectrometers became available using a prism to resolve the IR radiation into its component wavelengths. In the 1960s, the prism was replaced by a diffraction grating or a grating/prism combination, which allowed a substantial improvement in resolution. Dispersive IR spectrometers, however, suffer from several disadvantages, typically slow scan speed, poor wavelength accuracy/reproducibility and poor sensitivity. In the 1970's FTIR spectrometers, based on an entirely different principle, were developed. These instruments possess all the capabilities of grating IR spectrometers, but are faster, more sensitive and have unparalleled wavelength precision and accuracy. These factors, plus decreases in the capital cost of these instruments popularized FTIR instruments, which are have largely displaced mid-IR dispersive instruments in most laboratories.

2.3.1 Interferometry

FTIR spectroscopy is based on interferometry and thus differs fundamentally from traditional dispersive IR spectroscopy. The Michelson interferometer, used in most FTIR spectrometers, consists of a moving mirror and a fixed mirror situated perpendicular to each other (Figure 2.2).

A beamsplitter is employed to divide the beam of radiation from the IR source into two parts, one part being reflected to the moving mirror and the other being transmitted to the fixedg mirror. When the beams are reflected back, they recombine at the beamsplitter, producing a constructive/destructive interference pattern due to the difference in the distances traveled by the two components of the beam. After the IR

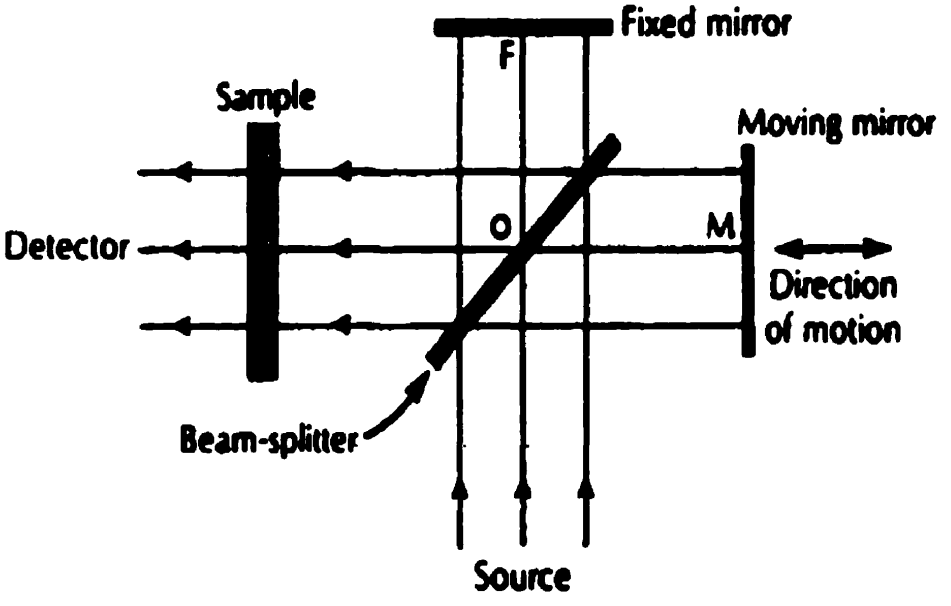

Figure 2.2. Schematic diagram of a Michelson interferometer. (6)

energy has been selectively absorbed by the sample, fluctuations in the intensity of the energy reaching the detector are digitized in real time, yielding an interferogram. The interferogram contains all the information that is required to produce the IR spectrum of the sample, however, this information is in the time domain. In order to obtain a conventional IR spectrum, the interferogram must be converted to the frequency domain by Fourier transformation. In FTIR spectroscopy, the interferogram is usually a plot of intensity as function of the path difference between the fixed and the moving mirror, known as retardation δ, which is proportional to time *t* because the moving mirror travels at constant velocity v, i.e.,

$$\delta = 2vt \text{ centimeters} \qquad [2.5]$$

Fourier transformation of the interferogram $I(\delta)$ then yields a spectrum with the x axis in units of wavenumbers (cm^{-1}), $I(\bar{\nu})$, in accordance with the following relationship:

$$I(\delta) = 0.5\, H(\bar{\nu})\, I(\bar{\nu}) \cos 2\pi\bar{\nu}\delta \qquad [2.6]$$

where $H(\bar{\nu})$ is a single wavenumber dependent correction factor that accounts for instrumental characteristics. Because carrying out a Fourier transform is computationally intensive, the development and application of FTIR spectroscopy has paralleled that of the personal computer, which facilitated the time to wavelength domain conversion as well as providing benefits related to spectral data manipulation and chemometrics.

2.3.2 FTIR Instruments

FTIR spectroscopy and its associated instrumentation is described in a number of excellent books (7-10), hence only the more pertinent aspects related to FTIR spectrometers are summarized. Commercial FTIR instruments first became commercially available in 1969, coming of age in the 1980s with the incorporation of chemometric software that made it practical to develop and implement IR calibrations. Although a variety of instrumental designs exist, all FTIR instruments have specific elements in

common; including a continuous source of IR radiation, an interferometer, a detector and a computer. The computer may control the optical components, collect and store data, perform signal averaging, carry out Fourier transforms and display the spectra. In addition, all FTIR instruments also have an internal reference laser to maintain wavelength accuracy.

Common IR sources used in the mid-IR include, Nernst glowers, Globars and nichrome coils, while for near IR, a tungsten lamp is the most widely used source. The Michelson interferometer is the most commonly used interferometer design and ideally, the beamsplitter is capable of precisely reflecting and transmitting 50% of the source radiation. In practice, most IR transparent materials used as beamsplitter material require a coating that only allows this ideal to be approximated. Thin films of germanium or silicon deposited on cesium iodide for bromide, sodium chloride, or potassium bromide beamsplitters are widely used for the mid-infrared region, while for the NIR a film of iron (III) oxide deposited on calcium fluoride is generally used (11).

There are two classes of IR detectors: thermal detectors and quantum detectors. Generally, thermal detectors are not readily adapted to FTIR instruments because of their slow response. Deuterated triglycine sulfate (DTGS) pyroelectric detectors are commonly used for the mid-IR region. For improved sensitivity and response, as well as high-resolution work, liquid-nitrogen-cooled mercury cadmium telluride (MCT) or indium antimonide photoconductive detectors are commonly employed (7,11). Lead sulfide detectors are commonly used in near-IR region while Indium gallium arsenide (InGAs) detectors are also used when greater sensitivity is required.

Compared to traditional dispersive instruments, FTIR instruments have fewer optical elements and no slits to attenuate radiation, resulting in higher energy throughput. FTIR instruments are also characterized by significant reductions in scan time, due to its multiplex advantage (Fellgett advantage), whereby all wavelength elements from the source reach the detector simultaneously, resulting in higher signal-to-noise (S/N) ratios. It is advantageous to measure all spectral elements simultaneously, since the detector

noise contribution is constant and is shared between all the spectral elements measured. Compared to near-IR, mid-IR sources are weaker and detectors can be relatively noisy, hence the NIR which combines stronger sources with more efficient detectors and are rarely limited by detector noise. Last, but not least is the incorporation of an internal HeNe laser in FTIR instruments, which is used to maintain a very high degree of wavelength accuracy, thus virtually eliminating wavelength drifts over time as a possible source of instrumental error, which can severely limit the accuracy of subsequent for spectral data manipulations and the precision of quantitative analysis.

2.4 Sample Handling Techniques

A wide range of sample types can be analyzed by IR spectroscopy, ranging from solids to gases that are well described in reference books covering this topic (9,12,13). In this research three sample handling methods were used: (a) a specialized oil transmission cell accessory, (b) a transmission vial accessory and (c) an attenuated total reflectance (ATR) accessory.

2.4.1 Transmission Cell Accessory

Transmission cells are the most common means of handling liquid sample for IR spectroscopy. Figure 2.3 illustrates the common elements making up a transmission cell, which may be demountable to allow one to vary the pathlength by varying the dimension of the spacer set between the windows. One of the two IR transparent windows is drilled to allow the liquid to be analyzed to flow into the gap between the windows via the syringe inlet/outlet ports. For quantitative work, such cells are commonly sealed so as to provide a stable, fixed pathlength.

The selection of the window material depends on the transmission range required as well as the IR characteristics of the sample to be analyzed. Table 2.3 lists the more commonly used IR window materials, their transmission characteristics and their relative water

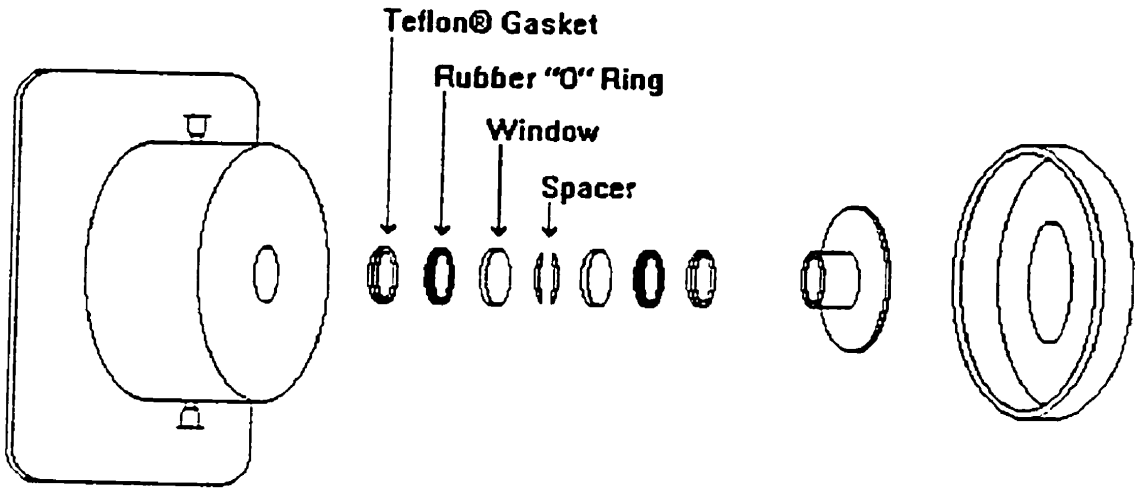

Figure 2.3. Schematic diagram of an infrared transmission cell (5).

Table 2.3. Properties of infrared window materials (5)

Material	Transmission range (cm^{-1})	Solubility in water	Refractive Index
Sodium Chloride(NaCl)	40,000-625	Soluble	1.49
Potassium Chloride (KCl)	40,000-500	Soluble	1.46
Potassium Bromide (KBr)	40,000-400	Soluble	1.52
Calcium Fluoride (CaF2)	66,666-1,100	Very slightly soluble	1.39
Barium Fluoride (BaF2)	50,000-870	Slightly soluble	1.42
Infrared quartz (SiO2)	50,000-2,500	Insoluble	1.74
Cesium Iodide (CsI)	10,000-200	Very Soluble	1.74
Thallium Bromide-Iodide (KRS-5)	15,385-250	Very slightly soluble	1.74
Silver Chloride (AgCl)	25,000-435	Very slightly soluble	1.98
Silver Bromide (AgBr)	20,000-285	Very slightly soluble	2.2
Irtran-2 (ZnS)	10,000-715	Insoluble	2.2
Zinc Selenide (ZnSe)	10,000-555	Insoluble	2.4
Germanium (Ge)	5,000-850	Insoluble	4.0

solubility. In the mid-IR region, when moisture is not an important consideration, low cost KCl and NaCl windows are widely used, while rugged quartz windows, and even glass, are commonly employed in near-IR analysis. Pathlength selection depends on the strength of the bands of interest and is adjusted by changing the thickness of the spacer placed between two windows. In mid-IR region, where absorptions are relatively strong, pathlengths ranging from 0.015-0.025 mm are common for liquids, while in the NIR longer pathlengths (1-5 mm) are common due to the weaker absorptions.

Although a general flow cell of the type illustrated in Figure 2.3 is useful for basic experimental work, the McGill IR Group has designed a specialized accessory for the analysis of fats and oils which is amenable to "at- or on-line" analyses. Figure 2.4 illustrates a general view of the accessory's cell and housing, which is manufactured by Dwight Analytical Solutions Ltd. (Toronto, Ontario, Canada). The housing is composed of a temperature controlled cell block and a removable cell insert. The cell insert can be removed or fixed in place using two screws, allowing one to readily change cells or take an open beam background. Vacuum is used to load and empty the cell and a three way valve is employed to control the flow of the sample through the cell or through a cell bypass line, allowing efficient removal of any previous sample from the larger volume lines without passing all the material through the cell as well as reducing cross contamination. The temperature control feature of this accessory is important in relation to the analysis of fats as a constant, elevated temperature is required to keep samples in a liquid state during the analysis. This accessory and more advanced, automated versions of it have been used for developing automated FTIR methods for the analysis of fats and oils (15-18).

2.4.2 Transmission Vial Accessory

In the NIR, quartz is a common window material, rugged, but relatively costly, either as a flow cell or cuvette. Glass is an inexpensive alternative and is readily available in the form of relatively consistent pathlength vials. Bomem has pioneered the use of

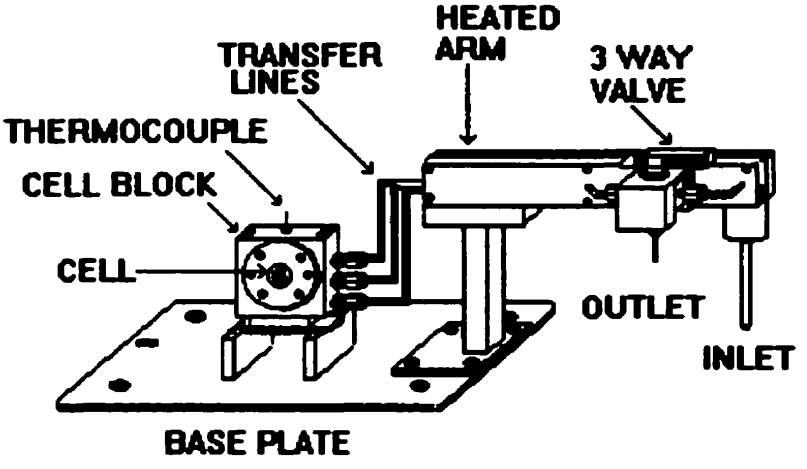

Figure 2.4. Oil analysis accessory, illustrating its component parts (14).

inexpensive glass vials as a means of sample handling in the NIR, having designed a temperature controllable multi-vial holding block (Figure 2.5) in which vials can be stored and analyzed.

The vial holder, depending on the diameter of the vial used, holds several to many vials, of which only one can be in the IR beam, while balance are conveniently stored in the block, which are equilibrating to the analysis temperature if the accessory is being heated. The advantage of this sample handling approach is that there is almost no sample preparation is needed, the vials can be labeled and are readily available for future reference and re-analysis if necessary. The main disadvantage of this sample handling system is that there is some variability in pathlength and curvature from vial to vial, however, these issues can be addressed by spectral normalization routines.

2.4.3 Attenuated Total Reflectance Accessory

Total attenuated reflectance (ATR) is based on the attenuation of a totally internally reflected IR beam by a sample placed in contact with a high reflectance index material known as internal reflection element (IRE). A detailed description of the principles of ATR spectroscopy was published in a book by Harrick (19) and a recent review of the applications of ATR to the analysis of food, including oils, has been written by Sedman *et al.* (20). The essential part of ATR accessory is a crystal of infrared transparent material having a high refractive index. Typical materials used as ATR elements include zinc selenide (ZnSe), KRS-5 (thallium iodine/thallium bromide), and germanium. Table 2.4 lists the properties of the more commonly used ATR materials.

When IR radiation strikes on the surface of high reflectance index materials above its critical angle, instead of partial reflection and partial refraction, total internal reflection occurs at the surface. The IR radiation undergoes multiple internal reflection as it travels down the crystal, giving rise to an evanescent wave at the surface of IRE. A sample brought into contact with the crystal can interact with the evanescent wave, absorb IR radiation from it and the FTIR spectrometer, via its detector generates a conventional IR spectrum. The evanescent wave decays exponentially as it propagate away from the

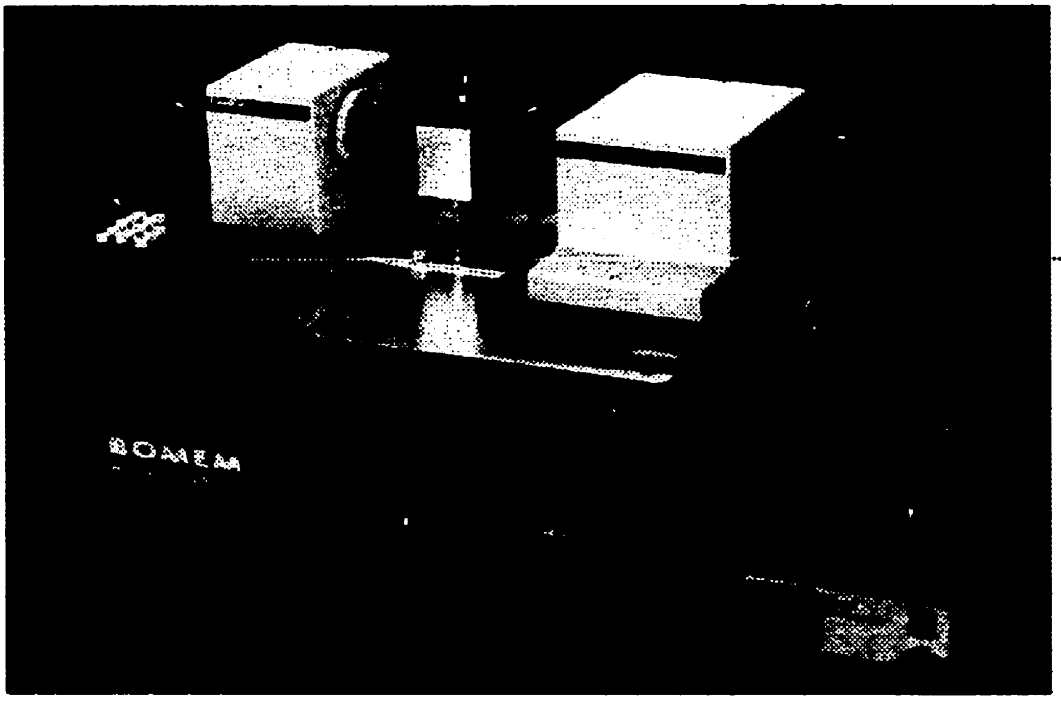

Figure 2.5. The Bomem MB-Series FT-NIR spectrometer with temperature-controlled multi-vial holding block.

Table 2.4. Properties of common ATR crystal materials (8,21)

Material	Crystal RI	Range(cm^{-1})	Comments
KRS-5	2.35	20,000-250	Deforms under pressure, easily scratched, toxic, slightly soluble in water
ZnSe	2.42	20,000-600	Hard, brittle, attacked by acids and strong alkalis
Si	3.42	8300-660	Hard, brittle, inert
Ge	4.0	5500-600	Hard, brittle, temperature sensitive transmission range, opaque above 120°C

surface of the IRE through the sample (Figure 2.6) and the depth of penetration is attenuated to 1/e of its total intensity. The effective pathlength (EPL) of ATR crystal is defined by depth of penetration multiplied by the reflections it undergoes while traversing the crystal.

ATR elements are most useful for samples with strong absorbtion bands, where short cell pathlengths become limiting, as the pathlengths of ATRs are inherently short (0.5-50 um). The depth of penetration, and hence the EPL of an ATR crystal is dependent on wavenumber, decreasing with frequency, hence the resulting spectra have different relative peak intensities than conventional transmission spectra. In addition, the crystal material, its length and thickness, as well as the angle of incidence also affect the effective pathlength. Since samples are placed on the crystal surface, sample handling is very straightforward for liquids and ATR can also be used to obtain the spectra of solids. Overall, a fair degree of latitude is available in dealing with general or particular applications as a wide variety of ATR sampling accessories are now commercially available, including fixed-angle and variable-angle types, single bounce (SB) and multiple bounces types, and flow cells, many having a temperature control option if needed. SB-HATR has recently become the accessory of choice for *trans* analysis for the Association of Official Analytical Chemists (22)

2.5 Chemometrics

In order to carry out quantitative analysis, one of the key assumptions is that the concentration of the constituents of interest in the samples are related to the data obtained by the measurement technique used to analyze them. The ultimate goal is to create a calibration equation (or series of equations) which, then applied to data of "unknown" samples measured in the same manner, will accurately predict the quantities of the constituents of interest. Since IR spectroscopy is generally a secondary method of analysis, development of a quantitative method requires calibration using a set of standards of known composition, either prepared gravimetrically or pre-analyzed by a primary chemical method to allow one to establish a relationship between IR band

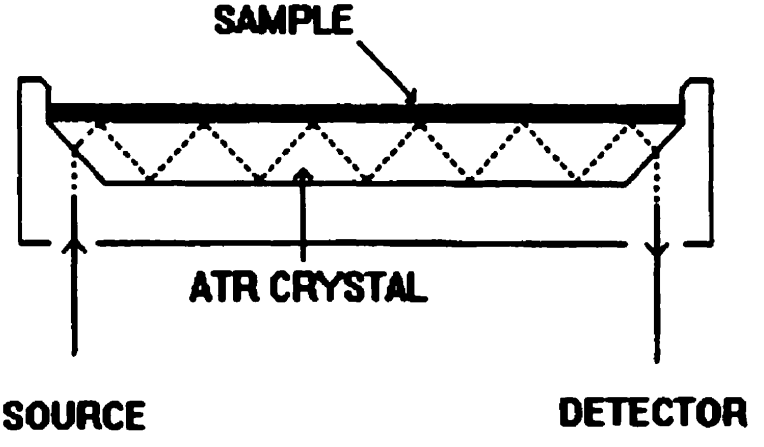

Figure 2.6. Schematic drawing of a horizontal ATR sampling accessory illustrating the internal reflection of the beam and its interaction with the sample on the surface of the crystal (20).

intensities and the compositional variable(s) of interest, usually concentration. Once a calibration has been established, it can be used for the prediction of the concentration of unknowns. However, in order for these predictions to be accurate, they must be measured under exactly the same conditions on the same instrument as the calibration set. Furthermore, the composition of the calibration standards has to be representative of the unknowns.

The mathematical approaches used to develop calibrations are generally referred to as "chemometrics" and the science of chemometrics gives spectroscopists a variety of tools by which quantitative methods can be devised from the analysis of spectral data. Each mathematical approach, whether simple or complicated, has its own advantages and disadvantages, the basic theory and application of chemometric methods having been the subject of a number of books and articles (14,23-27). The following section will provide a brief overview of the key approaches, ranging from basic Beer's law to more complicated multivariate methods including classic least squares (CLS), inverse least squares (ILS), principal components analysis (PCA) and partial least squares (PLS).

2.5.1 Beer's Law

The Beer-Lambert law, more commonly known as Beer's law, forms the basis of nearly all other chemometric methods in use for the analysis of spectroscopic data. It defines a simple linear relationship between concentration of an absorbing constituent in a sample and its absorption band in a spectrum:

$$A_\lambda = \varepsilon_\lambda b C \qquad [2.7]$$

where A_λ is the sample's absorbance value at specific wavelength (or frequency) λ, ε_λ is the absorptivity coefficient of the constituent at that wavelength, b is the pathlength through the sample and C is the concentration of the absorbing component of interest. The absorptivity coefficient is distinct from compound to compound and from wavelength to wavelength, however, it is constant for a given compound at a selected

wavelength. It can be determined by measuring the absorbance of a sample containing a known concentration of a constituent, or more precisely by preparing and measuring a series of standards containing different concentrations of the constituent, spanning the concentration range of interest so as to average out of random errors. Although Beer's law is simple and straightforward, it cannot account for any absorbance contributions due to other constituents which absorb at the wavelength of interest (A_λ) nor can it model interactions between constituents. Overlapping bands and component interactions are the more often the norm than the exception, and hence there is often a need to analyze for more than one component. In such cases Beer's Law becomes limiting and more sophisticated mathematical approaches are required to obtain valid quantitative analyses results.

2.5.2 Multicomponent Quantitative Analyses

Multicomponent analyses are designed to determine the concentrations of several components at a time and are based on the additivity of Beer's law, that is that the absorbance at a specific frequency is the sum of the absorbance of all sample components absorbing at that frequency. The mathematical relationship(s) used to predict unknowns is similar to Beer's law above but expressed in matrix form, multicomponent analyses being classified as classical least squares (CLS), inverse least squares (ILS) and factor analysis (PLS) methods depending on how the matrices are formulated.

2.5.2.1 Classical Least Squares Method

Classical least squares (CLS), also known as K-matrix calibration, is based on the direct representation of Beer's Law in matrix form:

$$A_{lm} = K_{ln} C_{nm} \qquad [2.8]$$

where A is a matrix of spectral absorbances, K is the matrix of absorptivity constants and C is the matrix of constituent concentrations. The subscripts indicate the matrix dimensionality: l is the number of frequencies at which absorbance measurements are

made, *m* is the number of calibration standards, and *n* is the number of constituents in the sample mixture. CLS calibrations do not necessarily require wavelength selection. As long as the number of wavelengths exceeds the number of constituents, any number can be used, even the entire spectrum. Using more wavelengths tends to have an averaging effect on the solution, reducing the effects of spectral noise and other random errors. The main limitation of CLS is that this chemometric method can only be applied to systems where the concentrations of every constituent in the sample is precisely known and do not interact, otherwise, unknown (undefined) constituents or their interactions with the component of interest may result in erroneous results. In practice, it is often difficult to meet the criteria of defining all constituents in a sample or there being no interactions, and to overcome these limitations ILS is a more practical alternative approach.

2.5.2.2 Inverse Least Squares (ILS) Method

Inverse least squares (ILS), also known as P-matrix calibration, is based on the inversing Beer's Law to solve for concentration and expressing it in matrix form:

$$C = PA \qquad [2.9]$$

Here concentration is expressed as a function of the absorbances at a series of specified frequencies. In contrast to CLS, where absorbance at a given frequency is calculated as an additive function of the constituent concentration, the matrix of coefficients (P) using ILS can still be calculated even if the concentrations of the other constituents in the mixture are not known. This is an important practical advantage, however, the number of selected frequencies cannot exceed the number of training samples due to the dimensionality of the matrix equations. This limitation cannot be overcome simply by adding more training samples, as this can lead to colinearity. Colinearity occurs when the absorbances in a spectrum tend to change together as the concentrations of the constituents in the mixture change, causing the mathematical solution to become less stable with respect to each constituent. Another limitation with ILS is overfitting, which prevents adding more frequencies to the calibration model. Generally, adding more frequencies to the calibration model will increase its prediction accuracy, however, at some point, the

predictions will start to deteriorate, as spurious spectral noise starts to be modeled. Thus, the advantageous averaging effect, present in CLS, is greatly reduced when using ILS and careful frequency selection is a key to developing successful ILS calibration models.

2.5.2.3 Factor Analysis Methods

The third and most sophisticated chemometric method available for quantitative analysis is factor analysis. Factor analysis shares the advantages of CLS and ILS approaches, but overcomes most of their particular limitations. The main difference between factor analysis methods and K-matrix and P-matrix methods is that factor analysis does not build direct relationships between concentration and absorbance measurements at specified frequencies, but rather relates "variation spectra", to concentration. Variation spectra are compressed spectral elements produced by factor techniques. They are also termed spectral loadings, loading vectors, principal components or factors and represent the changes in the absorbances at all the wavelengths in the spectra. These can be used instead of raw spectral data to build a calibration model. Subsequently, the sample spectrum is reconstructed from the "variation spectra" by multiplying each by a different constant scaling factor and adding the results together until the new spectrum closely matches the unknown spectrum. The scaling constants used to reconstruct the spectra are called "scores". Since the "variation spectra" are related to the concentration of the constituents, the scores can be used to predict the concentration of the unknowns.

Principal component regression (PCR) and partial least squares (PLS) are two most widely used factor analysis methods. PCR is a two-step process, in the first step, principal component analysis (PCA) technique is used to calculate the variation spectra and the scores, and then the scores are regressed against the constituent concentrations. PCR does not require frequency selection, any number, even the whole spectrum can be used, and PCA data compression allows using inverse regression to calculate model coefficients, thus the model can calibrate only for constituents of interest. However, there is no guarantee PCA vectors directly correspond to constituents of interest. Like PCR, PLS combines the full spectral coverage of CLS with partial composition regression of ILS. However, PLS differs from PCR in that PLS actually uses the concentration information

during the decomposition process and is a one step process. The factors are directly related to constituents of interest rather than largest common spectral variations. By using PLS, the spectra containing higher constituent concentrations are weighted more heavily than those with low concentrations, and the objective is to get as much as concentration information as possible into the first few loading vectors.

Although both PCR and PLS have many advantages over CLS and ILS, its main limitation is choosing the appropriate number of loading vectors or factors. Vectors are calculated and added to the calibration model in the order of importance, either based on variance in PCA or by concentration weighted variance in PLS. Hence, overfitting is a common problem, where more vectors than are actually necessary are included in the calibration model resulting in modeling instrumental or random noise rather than changes in concentration. On the other hand, when the calibration model does not have enough vectors "underfitting" is possible. In the former case, the model is very sensitive to noise rather than concentration changes, while in the latter it is not adequately responsive to changes in concentration. Unfortunately, there is no clear demarcation of the transition from desired "constituent" vectors into "noise" vectors so as to avoid under or over-fitting. There are techniques that aid in selecting what would seem an appropriate number of factors, such as the leave-one-out cross-validation method. This is done by performing the calibration n times with n-1 standards with the nth standard being predicted as an unknown. The predicted residual error sum of squares (PRESS) is then calculated from the errors in the predictions obtained for all n standards predicted by performing n calibrations, this process termed a cross-validation. The PRESS values obtained are then plotted as a function of the number of factors employed in the calibration. Ideally this plot is a down-sloping asymptote and the point at which the number of factors does not reduce the PRESS value significantly is considered to be the optimal number of factors for the model.

Although all the chemometric methods discussed have uses for developing quantitative relationships between spectral data and concentration, in the case of NIR spectroscopy,

the diffuse nature of the spectra make it difficult to work with anything but the more sophisticated multivariate techniques, by and large PLS.

2.6 Edible Oil Analysis by FTIR Spectroscopy

Quality control analyses based on chemical and physical characteristics of fats and oils are commonly carried out in the edible fats and oils industry. A number of "official" analytical methods sanctioned by the American Oil Chemists' Society (AOCS) and/or the Association of Official Analytical Chemists (AOAC) are widely used. Most of these are traditional wet chemical methods, which are tedious, time consuming, and expensive. Furthermore, significant amounts of solvents and reagents are generally required for these methods, which are often both hazardous and environmental unfriendly. Substantial research has taken place over the last decade with the objective of replacing the more common chemical methods with FTIR instrumental methods.

The application of IR spectroscopy to fats and oils analysis dates back to the beginning of 20th century. In 1905, Coblentz published the first compilation of IR spectra, including the spectra of several fatty acids and vegetable oils, followed in 1920 by Gibson's a paper entitled "The infra-red spectra of vegetable oils" (28). In practical terms no further analytical progress was made in relation to fats and oils until the late 1940s and early 1950s. At this time IR spectrometers became commercially available as a result of technological advances spurred on by World War II. In 1959, a tentative quantitative IR *trans* analysis method was adopted by the AOCS as Official Method Cd 14-61(29). Most IR lipid work up to the late 1980's tended to be quite fundamental and related to molecular structure and identification of constituents. Quantitative analyses were considered, but the dispersive instruments were generally not well suited to the task and there was a lack of suitable and readily accessible chemometric tools. The introduction of FTIR spectroscopy in the late 1960s revolutionized and revitalized IR spectroscopy that had been eclipsed by other powerful techniques such as nuclear magnetic resonance (NMR) spectroscopy and gas chromatography (GC). However, in practical, analytical terms, quantitative FTIR spectroscopy did not emerge until the late 1980's when the combination of cheap computing power, new chemometric techniques and lower cost instrumentation made FTIR more affordable and available. As of 1990, a substantive

body of research has been carried out to develop the quantitative analysis of fats and oils and it has since become apparent that FTIR spectroscopy is likely to play an important role in quality and process control of fats and oils (30)

FTIR spectroscopy has been used for band assignment, the characterization of fats and oils, the detection of adulterations, the determination of iodine value (IV), saponification number (SN), *trans* content, free fatty acids and solid fat index (SFI) determination as well as monitoring oil oxidation. Several reviews have been published on edible oil analysis by IR spectroscopy (14,31-36) and the subsequent sections will focus on those aspects relevant to this thesis.

2.6.1 Mid-IR Fats and Oils Analysis

2.6.1.1 Band Assignments

In order to use IR spectra to analyze fats and oils qualitatively and quantitatively, in either the mid or near-IR, one needs to understand the spectral characteristics (band assignments) in relation to lipid structure and their functional groups as well as those of their decomposition products or constituents commonly present. In the mid-IR, fundamental bands are well resolved and can be assigned to functional groups of the components of fats and oils. The assignments of the major bands of fats and oils were established as a result of the fundamental studies of the IR spectra of fatty acids and esters and triglycerides carried out in 1950s (37-41). Figure 2.7 presents a typical ATR mid-IR spectrum of an edible oil with its corresponding band assignments tabulated in Table 2.5. The bands at the high frequency end of the spectrum (3650~3590 cm^{-1}) in Figure 2.7, but not listed in Table 2.5, are bands due to the absorption of hydroxyl groups. These include water (H-OH), alcohols (R-OH) and primary oxidation products, hydroperoxides (RO-OH) and their breakdown products that often accumulate in oils as they oxidize. Aside from the well-defined bands listed in Table 2.5, there are still some bands which are difficult to assign in the "fingerprint" region (1550~600cm^{-1}). Ahemed (42) and co-workers assigned the band at 1400 cm^{-1} to terminal methyl groups on the aliphatic chains.

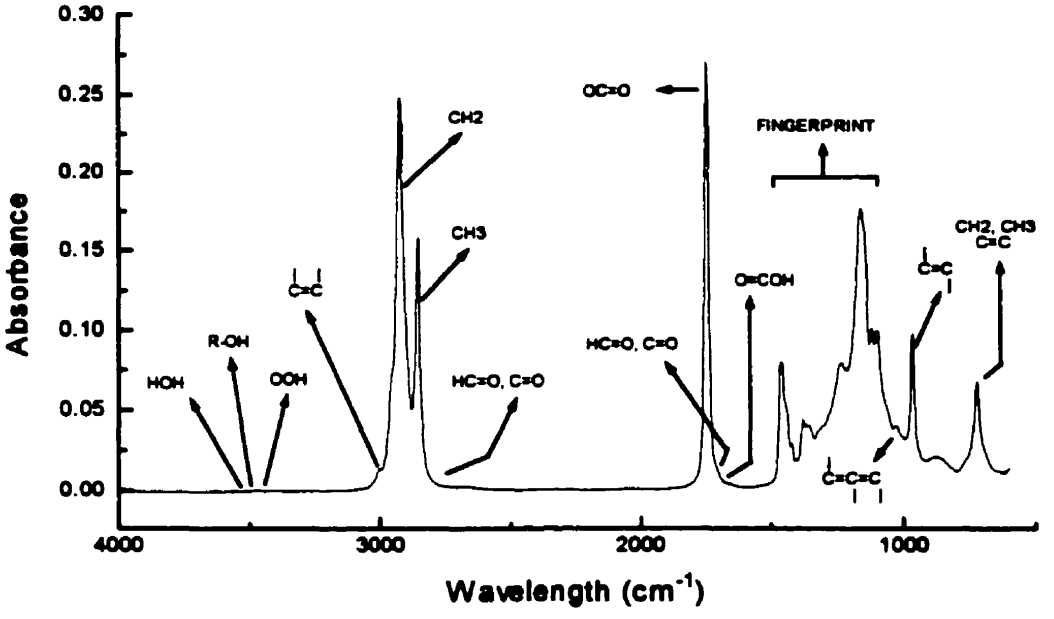

Figure 2.7. A typical ATR mid-IR spectrum of edible oil (45).

Table 2.5. Selected characteristic infrared absorption bands of vegetable oils

Band	Band Position (cm^{-1})	Assignment
1	3025	ν_{sym}(=C-H) (*trans* double bonds)
2	3008	ν_{sym}(=C-H) (cis double bonds)
3	2953	ν_{asym}(C-H) (CH_3 groups)
4	2925	ν_{asym}(C-H) (CH_2 groups)
5	2854	ν_{sym}(C-H) (CH_2 and CH_3 groups)
6	1746	ν(C=O) (ester linkage)
7	1666	ν(C=C) (*trans*)
8	1654	ν(C=C) (*cis*)
9	1459	CH_2 scissoring deformation
10	1161	ν(C-O)
11	967	Out-of-plane =C-H bending (*trans*)
12	722	CH_2 rocking

Absorptions of some bending vibrations of the methylene group are produced between 1350 and 1150 cm^{-1} (43). Sylverstein (4) and co-workers assigned bands between 1240and 1163 cm^{-1} to C-C(=O)-O band of saturated esters, and this band will shift to lower frequency in unsaturated esters. They also assigned the band between 1064 and 1031 cm^{-1} to O-C-C band of esters derived from primary alcohols, and this band will shift to 1100 cm^{-1} for the secondary alcohols. van de Voort and co-workers (15) related the band at 914 cm^{-1} to the bending vibration of *cis* disubstituted olefinic groups, while Wolf *et al.* (44) related it to vinyl groups.

Other than some of the more subtle absorptions in the fingerprint region which are still open to interpretation, fats and oils have generally been well characterized in the mid-IR.

2.6.1.2 Analytical Methods

Mid-IR spectroscopy, in particular FTIR spectroscopy, has been undergoing significant evolution in relation to quantitative analysis of fats and oils, being developed as an alternative to common AOCS chemical methods. As this thesis focuses on this aspect in relation to FT-NIR, the subsequent discussion summarizes literature relevant to the work undertaken.

2.6.1.2.1 Determination of Iodine Value

One of the most widely performed analysis in the fats and oil industry is the determination of the degree of unsturation or iodine value (IV), as it is an important indicator of the likely development of rancidity, as well as being an important hydrogenation process control parameter. IV is traditionally determined titrimetrically based on measuring the consumption of iodine after carrying out the reaction illustrated in equation [2.10]:

$$RHC{=}CHR' + I_2 \rightarrow RHIC\text{-}CIHR' \qquad [2.10]$$

the IV being expressed as the number of centigrams of iodine absorbed per gram of sample (29). The method is time consuming and uses hazardous chemicals, hence the industry would like to replace with a more efficient and environmentally friendly method. In 1971, Arnold and Hartung published work related to the determination of the degree of unsaturation of fats and oils by means of IR spectroscopy (46). Table 2.6 summarizes subsequent studies in terms of sample form, technique and their respective references.

As FTIR spectroscopy is our key concern, FTIR analysis for IV began with Afran (49) who modeled unsaturation using the peak height ratios of the C-H stretching vibration bands at 3010 and 2854 cm^{-1}. A mid-FTIR/ATR method for the direct prediction of IV was developed by van de Voort *et al.* (6). In this work, a PLS calibration model was developed to predict the IV based on 13 pure triglycerides used as calibration standards. As such, this method can be considered to be "universal" as it eliminates the need of chemical analyses of calibration standards. In 1995, van de Voort *et al.* (15) extended this approach to a customer designed flow through transmission flow accessory, considered more stable than the ATR accessories then available. In 1998, the flow cell method was validated by the independent analysis of more than 100 hydrogenated rapeseed and soybean samples (17) which resulted in excellent matches (within 1 IV unit) between FTIR PLS predictions and GC IV data. Recently, a method for the simultaneous determination of IV and *trans* content from the FTIR spectra of neat fats and oils recorded on a heated SB-HATR sampling accessory (52) has been developed. In this work, PLS regression was employed for the development of the calibration models, and a set of nine pure triacylglycerols served as the calibration standards, with good agreement (SD<1) obtained with GC reference data. Man *et al.* (50) developed a rapid FTIR method for determining palm oil IV using a PLS calibration covering the range of 3050 to 2984 cm^{-1}, which corresponds to the absorption band of =C-H *cis* stretching band. This method was demonstrated to be accurate (SD<0.151) and rapid (<2 min./sample) and they also compared various multivariate calibration techniques (51).

Table 2.6. Summary of methods investigated to determine the degree of unsaturation of fats and oils

Parameter	Sample	Technique	Reference
A_{3030}/A_{2857}	Dissolved	Dispersive	Arnold *et al.*, 1971 (46)
A_{1658}	Net	Dispersive	Bernard *et al.*, 1980 (47)
A_{3007}	Dissolved	Dispersive	Muniategui *et al.*, 1992 (48)
A_{3010}/A_{2854}	Neat	FTIR/ATR	Afran *et al.*, 1991(49)
PLS($A_{3200\text{-}2600}$ and $A_{1600\text{-}1000}$)	Neat	FTIR/ATR	van de Voort *et al.*, 1992 (6)
PLS($A_{3200\text{-}2600}$ and $A_{1600\text{-}1000}$)	Neat	FTIR/Cell	van de Voort *et al.*, 1995 (15)
PLS($A_{3200\text{-}2600}$ and $A_{1600\text{-}1000}$)	Neat	FTIR/Cell	Sedman *et al.*, 1998 (17)
PLS($A_{3050\text{-}2984}$)	Neat	FTIR/Cell	Man *et al.*, 1999 (50)
PLS($A_{3025\text{-}2992}$)	Neat	FTIR/Cell	Man *et al.*, 1999 (51)
PLS	Neat	FTIR/ATR	Sedman *et al.*, 2000 (52)

2.6.1.2.2 Determination of *trans* Content

In natural fats and oils, most unsaturated bonds are in their *cis* configuration. *Trans* isomers are commonly produced during hydrogenation to produce fats commonly used in the formulation of margarine, shortenings and other hardened fat products. *Trans* isomers are also found naturally in milk and beef fat as a result of hydrogenation in the rumen by microorganisms. *Trans* fatty acids are of increasing concern to nutritionists and the medical community due to their association with various metabolic disorders and heart disease (53-55). As a consequence, in late 1999, the U.S. Food and Drug Administration (FDA) tabled legislation to amend the nutrition labeling regulations to require the inclusion of *trans* fatty acids as part of the saturated fat component, together with a footnote reporting the *trans* fatty acid content. *Trans* content, previously only an industrial process control parameter, may become a legal labeling requirement in the near future and in effect force processors to have the ability to analyze for *trans* content.

Trans double bonds have strong absorptions over 980-965 cm^{-1} due to =CH deformation. The isolated *trans* double bond exhibits a characteristic, strong absorption band at 967 cm^{-1}, which forms the basis for the various IR methods for the determination of total and isolated *trans* contents. The development of analyses in relation to *trans* content has been reviewed by Ratnayake (56), Ulberth and Henninger (57), Firestone and Sheppard (58). The basic AOCS procedure for isolated *trans* determination (Cd 14-61) was introduced in 1946 (57) and was based on the use of a dispersive IR instrumentation. Carbon disulfide was used to dissolve triglycerides, fatty acids and fatty acids methyl esters and the transmission spectrum recorded over the region 1110 cm^{-1} to 910 cm^{-1}. The absorbance of the isolated *trans* double band at 967 cm^{-1} is measured after baseline correction and the % *trans* content determined by comparison with an elaidic acid analyzed under the identical conditions. Since its implementation, a number of approaches aimed at increasing the accuracy of the IR method have been suggested, some of which have been incorporated into newer revised methods, AOCS Cd 14-95 (59), AOAC Method 994.14 (60) and the IUPAC Method, 2.207 (61). Most modifications were aimed at correcting background interference (62-66) and there have been improvements to calibration and reference materials (67).

The introduction of FTIR and new sample handling systems, such as ATR and specialized transmission flow cells have also led to the improvement of *trans* determination. Computer aided FTIR, with its inherent advantages including better signal-to-noise ratios, high energy throughput and high wavelength accuracy, as well as the spectral ratioing capability, in combination with new chemometric techniques, have been successfully applied to determine isolated *trans* contents directly on neat oils. FTIR enables superior baseline correction, allows the elimination of CS_2, and eliminates the need to convert oil to its methyl esters.

Lanser and Emken (68) developed a FTIR method based on measurement of the area of the *trans* peak and obtained good agreement with of capillary GC. Sleeter and Matlock (69) developed a FTIR procedure for measuring the *trans* content of oils directly in their neat methyl ester form, eliminating the need for CS_2. Ulberth and Haider (70) used spectral subtraction to correct for sloping baselines, which enables the accurate measurement of low-levels of *trans*. They were also the first to use PLS chemometric procedures to quantitate *trans*, however, the analytical performance of their calibration was not validated. A more versatile PLS-FTIR method for simultaneous determination of *cis/trans* contents of neat edible fats and oils was developed by van de Voort *et al.* (15) in which pure triglycerides were used as calibration standards. This method was automated with a custom designed flow-through cell and the instrument programmed to automate the analysis procedure. One of the advantages of this method was that the calibration derived could be transferred between instruments, with no need to recalibrate from instrument to instrument. This work has been extended to simultaneously determine *cis/trans* content, IV and SN (16) and employs spectral ratioing technique facilitated by FTIR instruments. The single-beam FTIR spectrum of the fat or oil being analyzed was ratioed against the single-beam spectrum of similar reference oil that is free of *trans* groups. Thus the contributions of tryglyceride absorptions to the *trans* peak and the sloping background are eliminated. Mossoba *et al* (71,72) also demonstrated that a horizontal baseline was readily obtained by ratioing the single-beam spectrum of the sample against that of a reference material that is *trans* free using ATR, which requires only that a neat sample be poured onto the surface of the ATR crystal. One problem with this approach is that attention must

be paid to the selection of *trans* free oil which should be similar in composition to the samples to be analyzed. The precision of this method was found to be superior to those of transmission IR official methods by an international collaborative study (73). Spectral ratioing was adopted by the AOCS in 1996 as Recommended Practice Cd 14d-96 for the quantitation of isolated *trans* isomers at levels equal to or greater than 1% (59). Recently, a SB-FTIR method was developed for simultaneous determination of IV and *trans* content as mentioned in 2.6.1.2.1 (52), good agreement (SD<0.35) was obtained with AOCS FTIR/SB-HATR method. Another sample handling technique, the use of a disposable IR card was also studied in its application in *trans* determination (74) indicating that comparable results could be obtained using this technique.

2.6.1.2.3 Saponification Number

Saponification number (SN) is related to the average molecular weight of the triglycerides in fats and oils and is determined by acid/base titration after saponification (hydrolysis) of the triglycerides into glycerol and free fatty acids:

$$3\text{-}CH_2OCOR + 3KOH \rightarrow 3\text{-}CH_2OH + 3RCOOK \qquad [2.11]$$

with the saponification number being defined as the number of milligrams of potassium hydroxide required to saponify 1g of sample (28). The saponification number is indicative of its source and is used to monitor changes in interesterification processes, to monitor lipid fractionation and is an integral analysis for the determination of hydroxyl number of mono-diglyceride emulsifiers. An ATR/FTIR method was developed by van de Voort *et al.* (6) to determine the SN using a PLS calibration based on pure triglycerides, this concept also having been incorporated in a subsequent transmission method (15).

2.6.1.2.4. Determination of Peroxide Value

Under aerobic conditions, autoxidation is a major deteriorative reaction which takes place in edible oils adversely affecting their organoleptic and functional properties. Peroxide Value (PV) expressed as milliequivalents of peroxides per kilogram of oil is often used to

assess the oxidative stability of fats and oils, there being two AOCS methods available, Cd 8b-90 and Cd8-53 (29). Both methods, which differ in the solvents used, are based on the stoichiometric conversion of KI to molecular iodine by hydroperoxides in an acetic environment and subsequent titration of iodine with standardized sodium thiosulfate to determined the amount of the molecular iodine released.

$$ROOH + 2H^+ + 2I^- \rightarrow I_2 + ROH + H_2O \quad [2.12]$$

$$I_2 + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I^- \quad [2.13]$$

PV determination by FTIR spectroscopy has taken two tacks, one based on measurements related to the absorption of the O-H stretching of the hydroperoxide group and the other based on IR measurement of products formed when reagents react with hydroperoxides stoichiometrically. The first approach, which uses the ROO-H stretching absorption around 3550 cm^{-1} (75) is workable, but problematic due to hydrogen bonding, overlapping O-H stretching absorptions from alcohols and interference from the overtone of the ester carbonyl band. van de Voort *et al.* (76) was able to obtain a calibration using PLS, however, the calibration process was considered too complex for practical industrial implementation. A rapid FTIR method was developed by Moh and co-workers (77) to measure PV in crude palm and crude palm kernel oil. Calibration standards were prepared by oxidizing crude palm oil in a fermentor at 60°C over a period of 24 hours and the PLS calibration was built based on the 3710-3210 cm^{-1} spectral region with a single point baseline at 3710 cm^{-1}. The PV could be predicted by this method to within 1.5 PV. This group also developed a FTIR method for PV determination in thermally oxidized palm olein and the FTIR method was found to be better than that of the chemical method (78).

To overcome the complexity of the calibrations associated with direct measurements of –OOH, Ma *et al.* (79) developed a primary FTIR spectroscopic method for PV determination based on the stoichiometric reaction of triphenylphosphine (TPP) with hydroperoxides to produce triphenylphosphine oxide (TPPO). Accurate quantitation of the TPPO formed in this reaction by measurement of its intense absorption band at 542 cm^{-1} provides a simple means of determining PV. They extended this method from a

transmission cell to a 3M IR card (80). Due to unanticipated card fringing in the region where the measurements were normally made, the calibration based on the TPPO peak height at 542 cm^{-1} failed. However, the development of a PLS calibration eliminated the interfering effect of the fringes and allowed the TPPO band to be measured accurately.

2.6.2 NIR Fats and Oils Analysis: Band Assignments and Analytical Methods

Unlike mid-IR, there are no characteristic peaks for a given compound or functional group at a selected wavelength in the NIR, rather there is more or less a continuum of overlapping bands which represent largely C-H overtones and combination bands. As shown in Figure 2.8, the NIR can be roughly divided into the first (6200-5300 cm^{-1}) and second (8900-8000 cm^{-1}) overtones of C-H stretching vibrations, as well as the combination band regions 4900-4500 cm-1 and 7400-6700 cm^{-1} due to combinations involving the C-H stretching vibrations and other H vibration modes. Relatively little work has been done on fats and oils in the NIR, however, the spectra of free fatty acids were studied by Holman and Edmondson (81) who assigned the absorptions at 5952 (1680 nm), 4651(2150 nm) and 4562 cm^{-1} (2190 nm) to vibrations of C-H bonds bound to *cis*-unsaturation. Murray (82) found that fatty acids have characteristic spectral patterns around 5882 to 5780 cm^{-1} region and NIR absorption bands around 6250-5555 cm^{-1} (1600-1800 nm) and 4762-4545 cm^{-1} (2100-2200 nm) are assigned to the straight carbon chain and *cis* double bonds, respectively. In 1958, Holman *et al.* (83) identified the bands at 6849 and 4831 cm^{-1} (1460 and 2070 nm) as characteristic of hydroperoxides and assigned them to the overtone of the OO-H stretching absorption and combination absorption involving this vibration mode.

The lack of explicit spectral information associated with neat lipids has limited the utility of NIR. FT-NIR is a relatively new form of NIR instrumentation which can be considered an enhancement of conventional dispersive and filter based NIR instrumentation. The advantages of implementing FTIR spectroscopy to the NIR region of the spectrum are not as great as in the mid-IR, as the NIR has more energy available and better detectors. There are however still gains overall, specifically, in terms of wavelength accuracy, which improves the calibration development and performance characteristics.

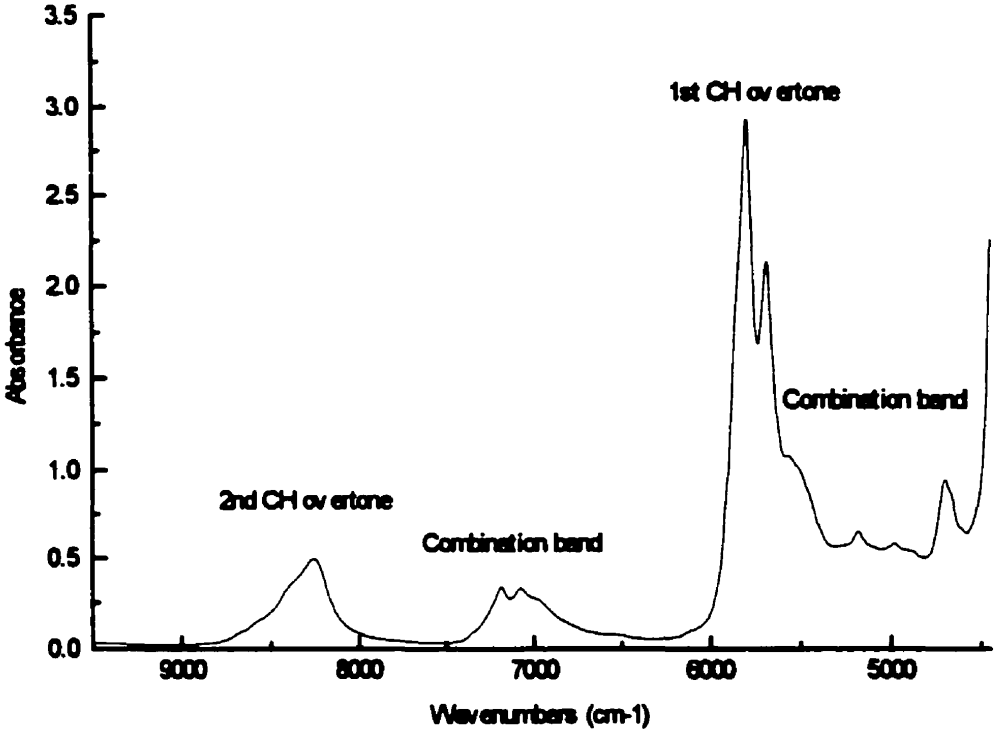

Figure 2.8. FT-NIR spectrum of a vegetable oil in a 8 mm (o.d.) glass vial.

In terms of fats and oils analysis, NIR spectroscopy has been used mostly to characterize and classify various of fats and oils and to detect oil adulteration, two important issues in the industry. NIR spectroscopy in conjunction with discriminant analysis has been used to differentiate between vegetable oils (84) and PCS was applied to classify nine vegetable oils (85) based on their PCA scores. The feasibility of identifying adulterants in extra virgin olive oil by NIR spectroscopy with the use of discriminant analysis has been demonstrated and adulteration could be measured to within ±0.9% (86,87). Holman *et al.* (81) related absorbance at 4651 cm^{-1} to the iodine value of fat while Wetzel (88) investigated the relationship between oil structure and NIR response. Sato *et al.* (89,90) studied NIR patterns in relation to fatty acid composition and Hong *et al.* (91) suggested that NIR spectroscopy could potentially serve as a nondestructive method for the rapid determination of palmitic, oleic and linoleic acid contents of oils.

Booth *et al.* (92) used NIR to monitor oil oxidation in used frying fats and oils, finding it suitable for the determination of free fatty acid content, dimer and polymer triglycerides. Hong *et al.* (93) determined PV in edible oils and established a multiple regression equation with a standard error of prediction (SEP) of PV of 1.098 meq/kg. Takamura *et al.* (94) also employed NIR spectroscopy to determine lipid oxidation and found that the intensity of the peak at 4798 cm^{-1} was highly correlated to peroxide value. Two wavelength (4807 and 4950 cm^{-1}) were selected by Cho and co-workers to build the calibration to predict PV of rancid soybean oil (95) and the SEP was 9.67 meq/kg oil. PLS was used by Moh *et al.* (96) to build a calibration model for crude palm oil PV determination, the calibration using 7407-6756 cm^{-1} with a single point baseline at 6605 cm^{-1}, with the predicted results being compatible with chemical method (SD<0.17). A FT-NIR method was also developed by Dong *et al.* (97) for the determination of PV based on stoichiometric reaction of TPP with hydroperoxides to form TPPO. Unlike the mid-IR method of Ma (79), TPP and TPPO absorptions overlapped extensively in NIR, however, with PLS, the TPPO formed could be measured accurately. Ismail *et al.* (98) investigated the suitability of FT-NIR spectroscopy as a technique for the bulk characterization of oils through the measurement of IV, *cis* and *trans* content. Their work indicated that FT-NIR spectroscopy, combined with PLS, could be suitable for *cis*, *trans*

and IV determination. They related the absorption at 8563 cm^{-1} to the *cis* double bond and a shoulder at 8503 cm^{-1}, not resolvable with traditional dispersive NIR spectrometers, to the *trans* double bond, however, further refinement of the calibration model was needed to obtain more accurate predictions.

2.7 Conclusion

Over the past 10 years, extensive research has been carried out by McGill IR Group and other researchers into the development of mid-FTIR methodology for the analysis of fats and oils. As a result, a variety of mid-IR methods have been developed, demonstrating the potential of FTIR spectroscopy as useful analytical quality control tool for the rapid analysis of fats and oils. However, mid-IR instrumentation, largely designed for a research environment, is generally not rugged enough for operation in an industrial setting, where it would be susceptible to vibration, temperature fluctuations and humidity. NIR instrumentation, traditionally designed for industrial environments is well accepted by process engineers and quality control departments. Many of the mid-FTIR methods developed, although of interest to the edible oils industry, falter due to sample handling issues and/or the lack of robustness of the instrumentation. ABB Bomem, a manufacturer of FT-NIR instruments recognized the market potential of IR analysis as applied to fats and oils, however, quantitative methodology was lacking. It was their contention that what can be done by mid-FTIR spectroscopy should be possible by FT-NIR spectroscopy and they sought out the McGill IR Group as a partner to carry out the fundamental research to investigate and develop FTIR methods. This overview of the basics of FTIR instrumentation, sample handling, chemometrics and relevant prior research should provide adequate background and a context for the research results presented in succeeding chapters in relation to the new FT-NIR methods developed.

2.8 References

1. Osborne, B. G., T. Fearn and P. H. Hindle, Theory of near infrared spectrophotometry, in *Practical NIR Spectroscopy with Applications in Food and*

Beverage Analysis, Longman Scientific & Technical, Harlow, England, pp13-35 (1993)

2. Anonymous, Terminology and Definitions in Vibrational Spectroscopy, in *Laboratory Methods in Vibrational Spectroscopy*, (edited by H. A. Willis, J. H. van de Maas and R. G. J. Miller), John Wiley & Sons, Wiltshire, Great Britain, pp5 (1991)

3. Cross, A. D., Elementary Theory of Infra-red spectroscopy, in *Introduction to Practical Infra-red Spectroscopy*. Butterworths Scientific Publications, London, pp2-5 (1960)

4. Sylverstein, R. M., Bassler, G. C. and Morrill, T. C., *Spectrometric Identification of Organic Compounds*, 5th ed. John Willey & Sons Inc., Wiltshire, Great Britain (1991)

5. Ismail A. A., F. R. van de Voort and J. Sedman, Fourier Transform Infrared Spectroscopy: Principles and Applications, in *Instrumental Methods in Food Analysis*, (edited by J. R. J. Pare and J. M. R. Belanger), Elsevier Science B. V., The Netherlands, pp93-136 (1997)

6. van de Voort, F. R., J. Sedman, G. Emo, and A. A. Ismail, Rapid and Direct Iodine Value and Saponification Number Determination of Fats and Oils by Attenuated Total Reflecance / Fourier Transform Infrared spectroscopy, *J. Am. Oil Chem. Soc.* 69: 1118-1123 (1992)

7. Griffiths, P. R., and James A. De Haseth, *Fourier Transform Infrared Spectrometry*, Wiley, New York (1986)

8. Smith, B. C., *Fundamentals of Fourier Transform Infrared Spectroscopy*, CRC Press, Boca, Raton, FL (1996)

9. Coleman, P. B. (eds.), *Practical Sampling Techniques in Infrared Analysis*, CRC Press, Boca, Raton, FL (1996)

10. Mckenzie, M. W., Ed., Advances in Applied Fourier Transform Infrared Spectroscopy, Wiley, New York, NY (1988)

11. Skoog, D. A. and Leary, J. J., Principles of Instrumental Analysis, 4th ed. Saunders College pub. (1992)

12. Miller, R. G. J. and C. Stace (eds.), *Laboratory Methods in Infrared Spectroscopy*, Heyden and Sons, London (1979)

13. Ferraro, J. R. and K. Krishnan (eds.), *Practical FT-IR Spectroscopy: Industrial and laboratory Chemical Analysis*, Academic Press, New York (1990)

14. Sedman, J., F. R. van de Voort, and A. A. Ismail, Application of Fourier Transform Infrared Spectroscopy in Edible Oil Analysis, in *New Techniques and Applications in*

Lipid Analysis, (edited by R. E. Macdonald and M. M. Mossoba), AOCS PRESS, Champaign, Illinois, pp283-324 (1997)

15. van de Voort, F. R., A. A. Ismail and J. Sedman, A Rapid, Automated Method for the Determination of *cis* and *trans* Content of Fats and Oils by Fourier Transform Infrared Spectroscopy (FTIR), *J. Am. Oil Chem. Soc.* 72: 873-880 (1995)

16. Sedman, J., F. R. van de Voort, and A. A. Ismail, Upgrating the AOCS Infrared *trans* Method for Analysis of Neat Fats and Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 74: 908 (1997)

17. Sedman, J., F. R. van de Voort, A. A. Ismail and P. Maes, Industrial Validation of FTIR *trans* and Iodine Value Analyses, *J. Am. Oil Chem. Soc.* 75:33-39 (1998)

18. van de Voort, F. R., J. Sedman and A. A. Ismail, in *Instrumental Methods in Food Analysis*, (edited by R. E. Macdonald and M. M. Mossoba), AOCS Press, 1st Edition, pp283 (1997)

19. Harrick, N. J., *Internal Reflection Spectroscopy*, Wiley-Interscience, New York, NY (1967)

20. Sedman, J., F. R. van de Voort, A. A. Ismail, Attenuated Total Reflectance Spectroscopy: Principles and Applications in Infrared Analysis of Food, in *Spectral Methods in Food Analysis: Instrumentation and Applications*, (edited by M. M. Mossoba), Marcel Dekker, Inc., New York, NY, pp397-425 (1999)

21. Anonymous, *The Complete Guide to FT-IR*, Spectra Tech, Inc., Shelton, Connecticut (1999)

22. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th edn, AOCS, Champaign, IL (1997)

23. Haaland, D. M. and E. V. Thomas, Partial Least-Squares Methods for Spectral Analyses. 1. Relation to Other Quantitative Calibration Methods and the Extraction of Quantitative Information, *Anal. Chem.* 60:1193-1202 (1988)

24. Haaland, D. M. and E. V. Thomas, Partial Least-Squares Methods for Spectral Analyses. 2. Application to Simulated and Glass Spectral Data, *Anal. Chem.* 60:1202-1208 (1988)

25. Kaufmann, P., Chemometrics in Lipid Analysis, in *Advances in Lipid Methodology – One*, (edited by W. W. Christie), The Oily Press LTD, Alloway, Ayr., Scotland, pp 149-180 (1992)

26. Kramer, R., *Chemometric Techniques for Quantitative Analysis*, Marcel Dekker, Inc., New York, NY (1998)

27. Anonymous, *PLSplus/IQ User's Guide*, Galactic, New York, NY (1998)

28. Gibson, K. S., The Infrared Spectra of Vegetable Oils, *Cotton Oil Press*, 4:53 (1920)

29. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn, AOCS, Champaign, IL (1989)

30. van de Voort, F. R. and J. Sedman, FTIR Spectroscopy –The Next Generation of Oil Analysis Methodologies? *INFORM* (in press)

31. Kochhar, S. P. and J. B. Rpssell, Analysis of Oils and Fats Using Infrared Spectroscopy, *Int. Anal.* 1:23-26 (1987)

32. van de Voort, F. R., Fourier Transform Infrared Spectroscopy Applied to Food Analysis, *Food Research International* 25:397 (1992)

33. van de Voort, F. R., J. Sedman and A. A. Ismail, Edible Oil Analysis by FTIR Spectroscopy, *Lab. Rob. Autom.* 8:205-212 (1996)

34. Guillen, M. D. and N. Cabo, Infrared Spectroscopy in the Study of Edible Oils and Fats, *J. Sci. Food Agric.* 75:1-11 (1997)

35. Maruyama, T., Analysis of Edible Fats and Oils, *Nihon Yukagakkaishi* 48:1097-1108 (1999)

36. Ismail A. A., Antonio Nicodemo, J. Sedman, F. R. van de Voort and I. E. Holzbaur, Infrared Spectroscopy of Lipids: Principles and Applications, in *Spectral Properties of Lipids*, (edited by R. J. Hamilton and J. Cast), CRC Press LLC, Boca Raton, FL, pp235-269 (1999)

37. Shreve, O. D., Heether, M. R., Knight, H. B., and D. Swern, Infrared Absorption Spectra [Some Long-Chain Fatty Acids, Esters and Alcohols]. *Anal. Chem.* 22:1498-1501 (1950)

38. O'Connor, R. T., E. T. Field and W. S. Singleton, The Infrared Spectra of Saturated Fatty Acids with Even Number of Carbon Atoms From Caproic, C_6 (hexanoic) to Stearic, C_{18} (octadecanoic), and of Their Methyl and Ethyl Esters, *J. Am. Oil Chem. Soc.* 28:154-160 (1951)

39. Sinclair, R. G., A. F. McKay and R. N. Jones, The Infrared Absorption Spectra of Saturated Fatty Acids and Esters, *J. Am. Chem. Soc.* 74:2570-2575 (1952)

40. Sinclair, R. G., A. F. McKay and R. N. Jones, The Infrared Absorption Spectra of Unsaturated Fatty Acids and Esters, *J. Am. Chem. Soc.* 74:2578-2586 (1952)

41. Ahlers, N. H. E., R. A. Brett and N. G. McTaggart, An Infrared Study of the *cis* and *trans* Isomers of Some C_{18} Fatty Acids. *J. Appl. Chem.*, 3:433-443 (1953)

42. Ahemed, F. A., R. O. Osman, H. M. El-Nomany and S. S. El-Saadany, Infrared and Ultraviolet Spectra of Some Lipids of Different Structures, *Grasas y Aceites* 37:250-253 (1986)

43. Guillen, M. D. and N. Cabo, Usefulness of the Frequencies of Some Fourier Transform Infrared Spectroscopic Bands for Evaluating the Composition of Edible Oil Mixtures, *Fett/Lipid* 101:71-76 (1999)

44. Wolf, I. A. and T. K. Miwa, Effect of Unusual Acids on Selected Seed Oil Analysis, *J. Am. Oil Chem. Soc.* 42:208-215 (1965)

45. Ma, K., Peroxide Value and *trans* Analysis by FTIR Spectroscopy, thesis, pp23 (2000)

46. Arnold R. G. and T. E. Hartung, Infrared Spectroscopy Determination of Degree of Unsaturation of Fats and Oils, *J. Food Sci.* 36:166-168 (1971)

47. Bernard, J. L. and L. G. Sims, IR Spectroscopy for the Determination of Total Unsaturation, *J. Sci. Food Agric.* 36:1120 (1985)

48. Muniategui, S., P. Paseiro and J. Simal, Measurement of the Degree of Unsaturation of Edible Oils and Fats by Using Infrared Spectroscopy and Its Relationship with Iodine Value, *Spain Grasas Aceites* 43:1-5 (1992)

49. Afran, A. and E. J. Newbery, Analysis of the Degree of Unsaturation in Edible Oils by Fourier Transform Infrared / Attenuated Total Reflectance Spectroscopy, *Spectroscopy* 6:31-34 (1991)

50. Man, Y. B. Che, G. Setiowaty and F. R. van de Voort, Determination of Iodine Value of Palm Oil by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 76:693-699 (1999)

51. Man, Y. B. Che and G. Setiowaty, Multivariate Calibration of Fourier Transform Infrared Spectra in Determining Iodine Value of Palm Oil Products, *Malay. Food Chem.* 67:193-198 (1999)

52. Sedman, J., F. R. van de Voort and A. A. Ismail, Simultaneous Determination of Iodine Value and *trans* Content of Fats and Oils by Single-Bounce Horizontal Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 77:399-403 (2000)

53. Hanis, T., V. Zidek, J. Sachova, P. Klir and Z. Deyl, Effects of Dietary *trans*-fatty Acids on Reproductive Performance of Wister Rats, *Brit J Nutr* 61:519-525 (1989)

54. Mensink, R. P. and M. B. Katan, Effects of Dietary *trans* Fatty acids on High-density and Low-density Lipoprotein Cholesterol levels in Healthy Subjects, *New Engl J Med* 323:439-445 (1990)

55. Gurr, M., A Fresh Look at Dietary recommendations, *INFORM* 7:432-435 (1996)

56. Ratnayake, W. M. N., Analysis of *trans* Fatty Acids, in *Trans Fatty Acids in Human Nutrition*, (edited by J. L. SeBedio and W. W. Christie), The Oily Press, Dundee, Scotland, pp235-267 (1998)

57. Ulberth, F. and M. Henninger, Estimation of the *trans* Fatty Acid Content of Edible Oils and Fats: An Overview of Analytical Techniques, *Eur. J. Med. Res.* 1:94-99 (1995)

58. Firestone, D. and A. Sheppard, Determination of *trans* fatty Acids, in *Advances in Lipid Methodology*, (edited by W. W. Christie), The Oily Press, Alloway, pp273-322 (1992)

59. AOCS, *Official and Tentative Methods of the American Oil Chemists' Society*, (edited by V.C. Mehlenbacher), AOCS, Chicago, IL (1946)

60. AOAC, *Official Methods of Analysis of the Association of the Official Analytical Chemists International*, 16th Edition, (edited by P. Cunniff), AOCS International, Arlington, VA (1995)

61. IUPAC, *Standards Methods for the Analysis of Oils, Fats and Derivatives*, 7th Edition, (edited by C. Paquot and A. Hautfenne), Blackwell Scientific Publications, Oxford (1987)

62. Firestone, D. and M. De La Luz Villadelmar, Determination of Isolated *trans* Unsaturation by Infrared Spectrophotometry, *J. Assoc. Off. Anal. Chem.* 44:459-464 (1961)

63. Firestone, D. and P. Labouliere, Determination of Isolated *trans* Isomers by Infrared Spectrophotometry, *J. Assoc. Off. Anal. Chem.* 48:437-443 (1965)

64. Huang, A. and D. Firestone, Determination of Low Level Isolated *trans* Isomers in Vegetable Oils and Derived Methyl Esters by Differential Infrared Spectrophotometry, *J. Assoc. Off. Anal. Chem.* 54:47-51 (1971)

65. Huang, A. and D. Firestone, Comparison of Two Infrared Methods for the Determination of isolated *trans* Unsaturation in Fats, Oils, and Methyl Ester Derivatives, *J. Assoc. Off. Anal. Chem.* 54:1288-1292 (1971)

66. Madison, B. L., R. A. DePalma and R. P. D'Alonzo, Acurate Determination of *trans* Isomers in Shortenings and Edible Oils by Infrared Spectrophotometry, *J. Am. Oil chem. Soc.* 59:178-181 (1986)

67. Ratnayake, W. M. N. and G. Pelletier, Methyl Esters from a Partially Hydrogenated Vegetable Oil is a Better Infrared External Standard than Methyl Elaidate for The Measurement of Total *trans* Content, *J. Am. Oil Chem. Soc.* 73:1165-1169 (1996)

68. Lanser, A. C. and E. A. Emken, Comparison of FTIR and Capillary Gas Chromatographic Methods for Quantitation of *trans* Unsaturation in Fatty Acid Methyl esters, *J. Am. Oil Chem. Soc.* 65:1483-1485 (1988)

69. Sleeter, R. T. and M. G. Matlock, Automated Quantitative Analysis of Isolated (nonconjugated) *trans* Uaing Fourier Transform Infrared Spectroscopy Incorporating Improvements in the Procedure, *J. Am. Chem. Soc.* 66:121-127 (1989)

70. Ulberth, F. and H. J. Haider, Determination of Low Level *trans* Unsaturation in Fats by Fourier Transform Infrared Spectroscopy, *J. Food Sci.* 57:1444-1447 (1992)

71. Mossoba, M. M. and D. Firestone, New Methods for Fat Analysis in Foods, *Food Testing and Analysis*, 2:24-32 (1996)

72. Mossoba, M. M., M. P. Yurawecz and R. E. McDonald, Rapid Determination of the total *trans* Content of Neat Hydrogenated Oils by Attenuated Total Reflection Spectroscopy, *J. Am. Oil Chem. Soc.* 73:1003-1009 (1996)

73. Adam, M., M. M. Mossoba, and T. Lee, Rapid Determination of Total *trans* Fat Content by Attenuated Total Reflection Infrared Spectroscopy: an International Collaborative Study, *J. Am. Oil Chem. Soc.* 77:457-462 (2000)

74. Ma, K., F. R. van de Voort, J. Sedman and A. A. Ismail, *trans* Fatty Acid Determination in Fats and Margarine by Fourier Transform Infrared Spectroscopy Using a Disposable Infrared card, *J. Am. Oil chem. Soc.* 76:1399-1404 (1999)

75. Fukuzumi, K. and E. Kobayashi, Quantitative Determination of Methyl Octadecadienoate Hydroperoxides by Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 49:162-165 (1972)

76. van de Voort, F. R., A. A. Ismail, J. Sedman and G. Emo, Monitoring the Oxidation of Edible Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 71: 243-253 (1994)

77. Moh, M. H., T. S. Tang, Y. B. Che Man and O. M. Lai, Rapid Determination of Peroxide Value in Crude Palm Oil Products Using Fourier Transform Infrared Spectroscopy, *J. Food Lipids* 6:261-270 (1999)

78. Setiowaty, G., Y. B. Che Man, S. Jinap and M. H. Moh, Quantitative Determination of Peroxide Value in Thermally Oxidised Palm Olein by Fourier Transform Infrared Spectroscopy, *Malay. Phytochem. Anal.* 11:74-78 (2000)

79. Ma, K., F. R. van de Voort, J. Sedman and A. A. Ismail, Stoichiometric Determination of Hydroperoxides in Fats and Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil chem. Soc.* 74:897-906 (1997)

80. Ma, K., F. R. van de Voort, A. A. Ismail and J. Sedman, Quantitative Determination of Hydroperoxides by Fourier Transform Infrared Spectroscopy with a Disposable Infrared Card, *J. Am. Oil chem. Soc.* 75:1095-1101 (1998)

81. Holman, R. T. and P. R. Edmondson, Near-infrared Spectra of Fatty Acids and Some Related Substances, *Anal. Chem.* 28:1533-1538 (1956)

82. Murray, I., in *Near Infrared Diffuse reflectance/transmittance Spectroscopy*, (edited by J. Hollo, K. J. Kaffka and J. L. Gonczy), Akademiai Kiado, Budapest, pp13-28 (1987)

83. Holman, R. T., C. Nickell, O. S. Privett and P. R. Edmondson, Detection and Measurement of Hydroperoxides by Near Infrared Spectrophotometry, *J. Am. Oil Chem. Soc.* 35:422-425 (1958)

84. Bewig, K. M., A. D. Clarke, C. Roberts and N. Unklesbay, Discriminant Analysis of Vegetable Oils by Near-infrared Reflectance Spectroscopy, *J. Am. Oil Chem. Soc.* 71:195-200 (1994)

85. Sato, T., Application of Principal-Component Analysis on Near-Infrared Spectroscopic Data of Vegetable Oils for Their Classification, *J. Am. Oil Chem. Soc.* 71:293-298 (1994)

86. Wesley, I. J., R. J. Barnes and A. E. J. McGill, Measurement of Adulteration of Olive Oils by Near-Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 72:289-292 (1995)

87. Wesley, I. J., F. Pacheco and A. E. J. McGill, Identification of Adulterants in Olive Oils, *J. Am. Oil Chem. Soc.* 73:515-518 (1996)

88. Wetzel, D. L., Lipid Structures and NIRA, presented at the 4th International Symposium NIRA, Kansas States University, Manhattan (1983)

89. Sato, T., S. kawano and M. Iwamoto, Near Infrared Spectral Patterns of Fatty Acid Analysis from Fats and Oils, *J. Am. Oil Chem. Soc.* 68:827-833 (1991)

90. Sato, T., Application of near infrared Spectroscopy for the Analysis of Fatty Acid Composition, *Japan. Lipid Technol.* 9:46-49 (1997)

91. Hong, J., S. Yamaoka-Koseki and K. Yasumoto, Determination of Palmitic acid, Oleic Acid and Linoleic Acid by Near-infrared Transflectance Spectroscopy in Edible Oils, *Food Sci. Technol., Int.* 2:146-149 (1996)

92. Boot, A. J. and A. J. Speek, Determination of the Sum of Dimer and Polymer Triglycerides and of Acid Value of Used Frying Fats and Oils by Near-infrared Reflectance Spectroscopy, *J. AOAC Int.*, 77:1184-1189 (1994)

93. Hong, J., S. Yamaoka-Koseki and K. Yasumoto, Analysis of Peroxide Values in Edible Oils by Near-infrared Spectroscopy, *Japan. Nippon Shokuhin Kogyo Gakkaishi* 41:277-280 (1994)

94. Takamura, H., N. Hyakumoto, N. Endo, T. Matoba and T. Nishiike, Detrmination of Lipid Oxidation in Edible Oils by Near Infrared Spectroscopy, *J. Near Infrared Spectrosc.* 3:219-225 (1995)

95. Cho, S., J. Kim and C. Rhee, Determination of Rancidity of Soybean Oil by Near Infrared Spectroscopy, *J. Near Infrared Spectrosc.* 6(1-4): A349-A354 (1998)

96. Moh, M. H., Y. B. Che Man, F. R. van de Voort and W. J.W. Abdullah, Determination of Peroxide Value in Thermally Oxidized Crude Palm Oil by Near Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 76:19-23 (1999)

97. Dong, J., K. Ma, F. R. van de Voort and A. A. Ismail, Stoichiometric Determination of Hydroperoxides in Oils by Fourier Transform Near Infrared Spectroscopy, *J. AOCS Int.* 80:345-352 (1997)

98. Ismail, A. A., C. Charbonneau, J. Sedman and F. R. van de Voort, Comparative Analytical Performance of mid-FTIR and FT-NIR Spectroscopy in the Determination of *cis* and *trans* Content of Fats and Oils, *Book of Abstracts, Pittcon'96, Chicago, IL, March 3-8*, Abstract 012 (1996)

CHAPTER 3 RAPID DETERMINATION OF CIS AND TRANS CONTENT, IODINE VALUE, AND SAPONIFICATION NUMBER OF EDIBLE OILS BY FOURIER TRANSFORM NEAR-INFRARED SPECTROSCOPY

3.1 Abstract

Fourier transform near infrared (FT-NIR) spectroscopy was evaluated as a means of simultaneously determining the *cis* and *trans* content, iodine value, and saponification number of neat fats and oils. Reference values for these parameters were obtained from oils using a previously developed mid-FTIR Edible Oil Analysis Package. Two partial least squares (PLS) calibrations were developed for a 5mm heated flow cell, the first a process calibration based on hydrogenated soybean samples and the second a more generalized calibration based on an oil sample matrix containing many oil types and designed to remove any correlations among the parameters measured. Each calibration performed well with its own validation samples; however, only the non-correlated calibration was able to analyze oil samples accurately from a variety of sources. It was found that NIR analysis maintained the internal consistency between *cis/trans* and IV, and the accuracy and reproducibility of the predictions were in the order of ±1.5 and ±1.0 units, respectively, for all parameters evaluated. FT-NIR is shown to be a very workable means of determining *cis/trans*/IV values and saponification number for edible fats and oils, and it provides a rapid alternative to the commonly used chemical and physical methods presently employed in the industry.

Keywords: *cis* content, Fourier transform near-infrared spectroscopy, FT-NIR, iodine value, oil analysis, partial least squares, PLS, saponification number, *trans* content

3.2 Introduction

Some key factors which determine the chemical and physical properties of edible fats and oils include the relative degree of unsaturation or iodine value (IV), the type of unsaturation (*cis* or *trans*) and the weight-average molecular weight or saponification number (SN). Fats and oils commonly undergo processes such as hydrogenation, which modify the physico-chemical characteristics of fats and oils by changing the IV and

cis/trans ratio, for which specific values are often targeted. Hence monitoring changes in these parameters during a process is important as they define the quality and functionality of the end product. In addition, the *trans* content may become of potential regulatory interest because of its association with arteriosclerosis and heart disease (1) and may one day be a required analysis for labeling purposes. Although AOCS methods are available to measure these parameters, the official methods tend to laborious, and the industry is always on the alert for more rapid and efficient ways of carrying out routine analyses.

The McGill IR Group has focused on the development of rapid methods for edible oil analysis using Fourier transform mid-infrared (mid-FTIR) spectroscopy (2-5) specifically in relation to industrial quality control applications. A rapid, quantitative mid-FTIR method was developed to simultaneously determine *cis* and *trans* content, IV, and SN, the calibration being based on pure triglyceride standards and using partial least squares (PLS) as the chemometric approach (6). Although mid-FTIR transmission spectroscopy is gaining acceptance as an analytical tool in the edible oil sector, near infrared (NIR) spectroscopy has seen more use in industrial applications because NIR instruments are more rugged and less energy limited than mid-FTIR spectrometers, can use glass or quartz cells, and have sensitive detectors. In particular, NIR reflectance instruments are widely used by the food industry for the rapid quantitative determination of moisture, lipid, protein, carbohydrates and fiber in agricultural and food products (7). In the case of edible oils, NIR transmission techniques can be employed; they eliminate many of the difficulties associated with reflectance spectroscopy, especially in relation to calibration development and maintenance. A number of oil processors have expressed an interest in FT-NIR-based edible oil applications, in particular peroxide value, IV and *trans* analyses. An transmission FT-NIR oil analysis method for the rapid determination of peroxide value (8) has been described previously, and this paper presents the method development protocol, calibration and validation of an FT-NIR method for the simultaneous determination of *cis*, *trans*, IV and SN parameters of edible fats and oils.

3.3 Materials and Methods

3.3.1 Oil Samples, Calibration Standards and Validation Samples

Hydrogenated soybean and rapeseed oils with a wide variety of *cis/trans* and IV values were obtained courtesy of a major oil processor. Other oils, including coconut oil, soybean oil, olive oil, canola oil, corn oil and sunflower oil, were obtained from retail and commercial outlets. Trisun HB 95, a partially hydrogenated, high-*trans* (65%) sunflower oil (Lot #3R147) was obtained from SVO Specialty Products, Inc. (Eastlake, OH).

Two sets of calibration standards were employed in this work. The first set, comprising of 32 hydrogenated soybean oil samples and covering a wide range of *trans* values (~0-50% *trans*), was employed to develop a calibration designed to monitor a particular hydrogenation process. For the development of a universal calibration, 29 samples were prepared by mixing a variety of commercial fats and oils of varying *cis*, *trans*, IV and SN values, Trisun HB95 being used to extend the *trans* value range of the blends. These samples were combined with 11 commercial hydrogenated soybean and 11 hydrogenated rapeseed samples to produce a calibration set which covered a broader range of *cis/trans* IV and SN values than would be found in any single oil type as well as obviating *cis/trans* and IV intercorrelations. For validation of the two calibrations developed, hydrogenated rapeseed and soybean oils not used in either calibration set were employed as validation samples. The reference values for *cis* content (expressed as percent triolein), *trans* content (expressed as percent trielaidin), IV and SN for all the calibration standards and validation samples were obtained using the mid-FTIR Edible Oil Analysis Package (EOA Package) developed by the McGill IR Group and described in a previous publication (6).

3.3.2 Instrumentation and Sample Handling

FT-NIR spectroscopy was carried out using a Bomem FT-NIR spectrometer (Hartmann & Braun MB-Series, Bomem, Inc., Quebec) capable of covering the spectral range of 12,000-2000 cm^{-1}, controlled by an IBM-compatible 486 DX-66 MHz PC running under Windows-based Bomem-Grams/386 (Galactic Industries Co., Salem, NH) software. The instrument and optical path were purged with a continuous flow of dry air from a Balston

dryer (Balston, Lexington, MA) to minimize water vapor and CO_2 interferences. Basic spectroscopic work was carried out using a heated transmission flow cell accessory (5) with quartz windows and having a path length of 5mm; the system was capable of handling preheated fats and oils in their neat state (Dwight Analytical Solutions Ltd., Toronto, Ontario). The accessory was maintained at 80 ± 0.2°C to ensure that fats were liquefied, the samples being melted in a microwave oven prior to being loaded into the flow cell. FT-NIR spectra of the samples were collected over the range of 10,000-4000 cm^{-1}, an open beam emittance spectrum being collected first, followed by the collection of a sample emittance spectrum (128 co-added scans for each). These were then being ratioed and transformed into a conventional absorbance spectrum. Mid-FTIR spectroscopy was carried out using a Nicolet Magna FTIR spectrometer (Nicolet Instrument, Madison, WI), to obtain reference *cis/trans*/IV/SN data using the EOA Package. Data obtained from this system was used as reference values to calibrate the FT-NIR system and validate its predictions.

3.3.3 Calibration

The spectra of the calibration standards together with the *cis/trans*/IV/SN data obtained from the mid-FTIR EOA package were input into Omnic TQ Analyst chemometrics program (Nicolet Instrument Corp., Madison, WI) to develop NIR calibrations. Correlation spectra, which relate spectral changes to the values of the parameter of interest, as well as variance spectra, which illustrate regions of spectral variance in the standards, were generated. These were employed to assist in identifying spectral regions, which could be used to develop predictive PLS models for *cis* and *trans* content, IV and SN. The predicted residual error sum of squares (PRESS) test as well as the root mean square error (RMSE) associated with the cross validation of the calibrations tested were used to select optimal calibrations. The performance of the calibrations as well as the validations was assessed using linear regression, with accuracy and reproducibility assessed using mean differences (MD) and standard deviation of the differences (SDD) according to the method of Youden and Steiner (9).

3.4 Results and Discussion

3.4.1 General Considerations

NIR spectroscopy differs fundamentally from mid-IR spectroscopy, because the spectral profiles in the NIR region contain less spectral detail and consist of overlapping and poorly defined overtone and combination bands arising from the fundamental absorptions occurring in the mid-IR region. Most of the strong absorption bands in the NIR region are CH overtone and combination bands. The region covering 6200-5500 cm^{-1} comprises the first ν CH overtone, whereas 8500-8200 cm^{-1} is associated with the second ν CH overtone. CH_2 combination bands appear in the 4500-4000 cm^{-1} range. The absorbances near 8475 and 5900 $cm^{-1}$are due to strong -CH=CH- (*cis* double bond) overtones, the respective combination bands being found near 4657 and 4566 cm^{-1}(10). The *trans* overtone and combination bands are very weak and can be difficult to measure, tending to be overwhelmed by stronger absorptions. In terms of edible oils, Sato *et al.* (11) reported that most of the more obvious spectral differences in their NIR spectra are evident in the 6250-5555 cm^{-1} and 4762-4545 cm^{-1} regions. Holman *et al.* (12) related changes in absorbance at 4651 cm^{-1} to the IV of fats and oils, whereas Wetzel (13) correlated NIR absorbance changes at 5952, 4675 and 4529 cm^{-1} to solid-fat index, degree of unsaturation and SN, respectively. Although these citations indicate that absorptions at selected wavelengths correlate with various physicochemical properties of fats and oils, the absorption bands in the NIR region overlap so extensively that traditional univariate analysis techniques are generally not applicable. Because of this, it is mandatory to make use of advanced chemometric techniques such as PLS to obtain meaningful quantitative data. PLS is a sophisticated multivariate analysis technique that has largely been pioneered for NIR applications and has played a major role in the recent resurgence of quantitative mid-IR spectroscopy. The key difference between PLS and multiple linear regression approaches is that a PLS calibration does not entail establishing direct relationships between concentration and absorbance measurements at specified frequencies (i.e., peak heights or peak areas), but rather develops a model by compressing the spectral data for a set of calibration standards into a series of mathematical "spectra", known as loading spectra or factors. PLS decomposes the spectrum of each calibration

standard into a weighted sum of the loading spectra, and the weights given to each loading spectrum, known as "scores", are regressed against the concentration data for the standards. When the spectrum of an unknown is analyzed, PLS attempts to reconstruct the spectrum from the loading spectra, and the amounts of each loading spectrum employed in reconstructing the spectrum, i.e., the "scores", are then used to predict the concentration of the unknown (14). A PLS calibration can, in principle, be based on the whole spectrum, although in practice the analysis tends to be restricted to regions of the spectrum that exhibit variations with changes in the concentrations of the components of interest. As such, the use of PLS can provide significant improvements in precision relative to methods that use only a limited number of frequencies. In addition, PLS treats concentration rather than spectral intensity as the independent variable and is thereby is able to compensate for unidentified sources of spectral interference (i.e., overlapping bands), which are common in NIR spectroscopy.

Certain restrictions are inherent in the PLS method. First, any PLS calibration model will only give accurate predictions for samples that are well represented by the calibration standards. Second, in a multicomponent analysis the predictions obtained from a PLS model will reflect any intercorrelation that exists in the calibration set. Hence, for a generalized calibration it is essential that the values of all parameters measured vary independently. However, in developing a calibration for a specific product line, this may be neither necessary nor readily feasible, as the samples conveniently available for calibration may contain components whose concentrations are inherently correlated. In the present work, two types of calibration models were developed, one based on samples taken from an industrial soybean hydrogenation process and the other based on a wide variety of oil types. For the first calibration, analysis of the calibration standards by mid-FTIR spectroscopy demonstrated that the *cis* and *trans* contents are inversely related and highly correlated (Figure 3.1). In contrast, the second calibration was designed to have more variability in all parameters as well as to ensure that there were no intercorrelations between any pair of parameters (Figure 3.2). Both calibration approaches are valid, but they differ in the scope of application of the calibrations derived.

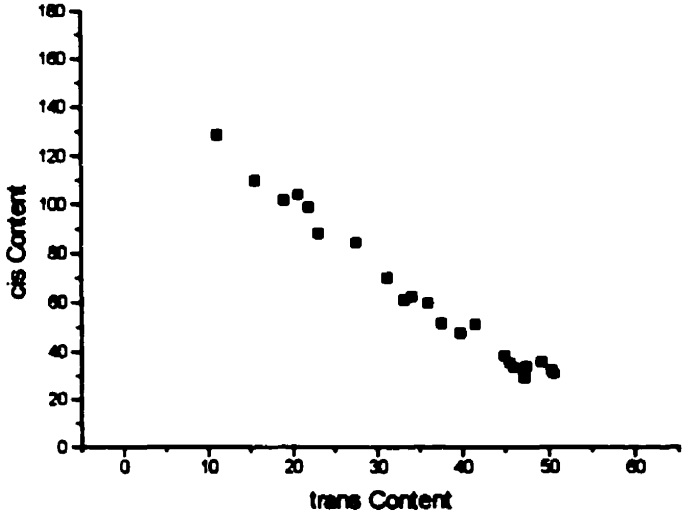

Figure 3.1. *Cis* and *trans* mid-FTIR values for the soybean process calibration set, illustrating their intercorrelation.

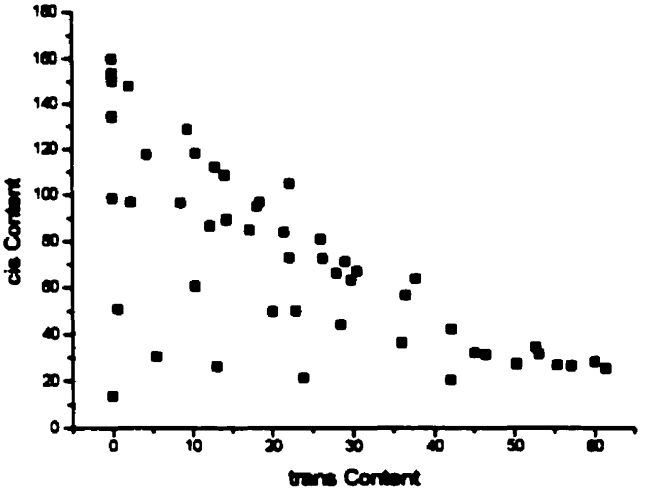

Figure 3.2. *Cis* and *trans* values of the generalised calibration set, illustrating how intercorrelation was avoided ensuring that these variables would be evaluated independently.

3.4.2 Development of a Calibration Based on Process Samples

Figure 3.3 illustrates the mean, variance, and correlation spectra for the calibration set, comprising samples taken from a soybean oil hydrogenation process. The *cis*, *trans* and IV correlation spectra strongly resemble each other because of the inherent correlation between the three parameters in question within this set of process samples. SN was not considered in this analysis, as it is effectively invariant.

The variance spectrum shows that there are three main regions in which there are significant spectral variations, 8700-8000 cm^{-1}, 6100-5350 cm^{-1} and 4900-4500 cm^{-1}, with some minor variations around 7200 cm^{-1}. The correlation spectra, which relate the changes in the values of each parameter to spectral changes, are more complex; however, the regions of high correlation correspond to those in which the most spectral variation is observed. These regions basically correspond to the first and second CH overtone regions (6200-5300 and 8900-8000 cm^{-1}) and the corresponding CH combination band regions (4900-4500 and 7400-6700 cm^{-1}). A number of calibrations were assessed using these regions and combinations thereof. On the basis of the RMSE of the predictions for a separate set of validation samples, the first combination band region encompassing 4777-4553 cm^{-1} using a single point baseline at 4800 cm^{-1} was found to produce good calibrations for all three parameters with a minimum predictive error. Figure 3.4 illustrates the one-to-one correspondence between the NIR IV predictions for a set of soybean validation samples and the mid-FTIR reference values. Similar validation plots were obtained for *cis* and *trans* content, and Table 3.1 summarizes the MDa and SDDa for the three parameters measured.

Another basis for evaluating the calibrations is to assess whether they are internally consistent, as there is a defined relationship (Equation [3.1]) between *cis*/*trans* content and IV, as has been discussed elsewhere (6):

$$IV = 0.8601cis + 0.8601trans \qquad [3.1]$$

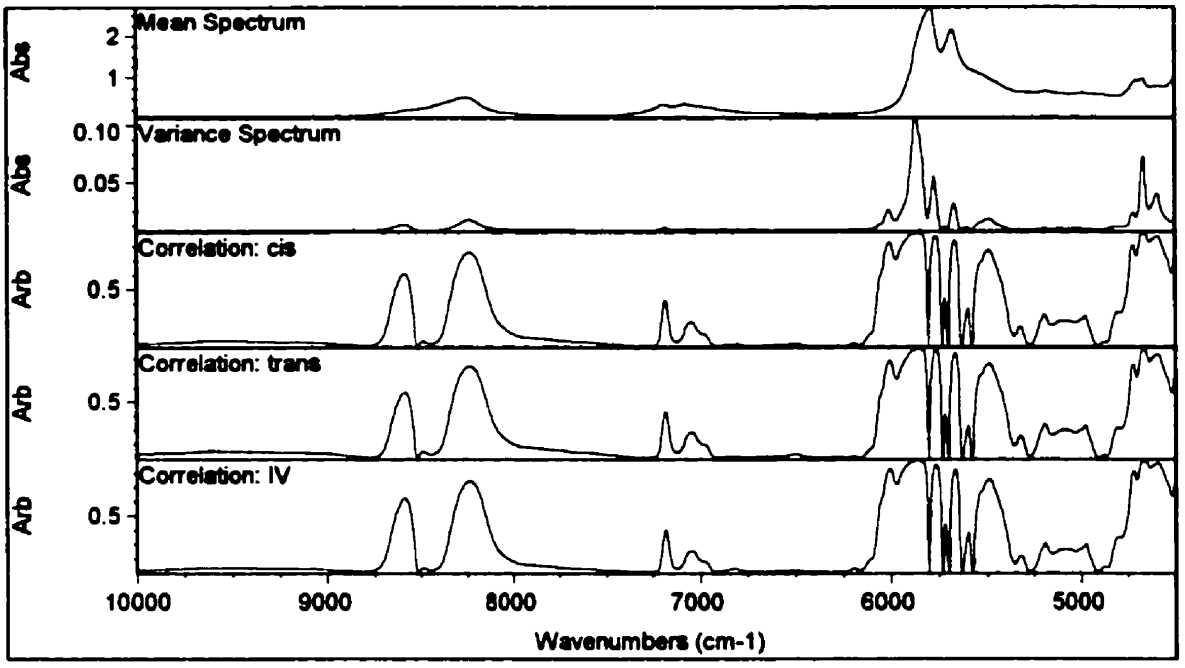

Figure 3.3. Mean, variance and *cis/trans*/IV correlation spectra of the soybean calibration standards.

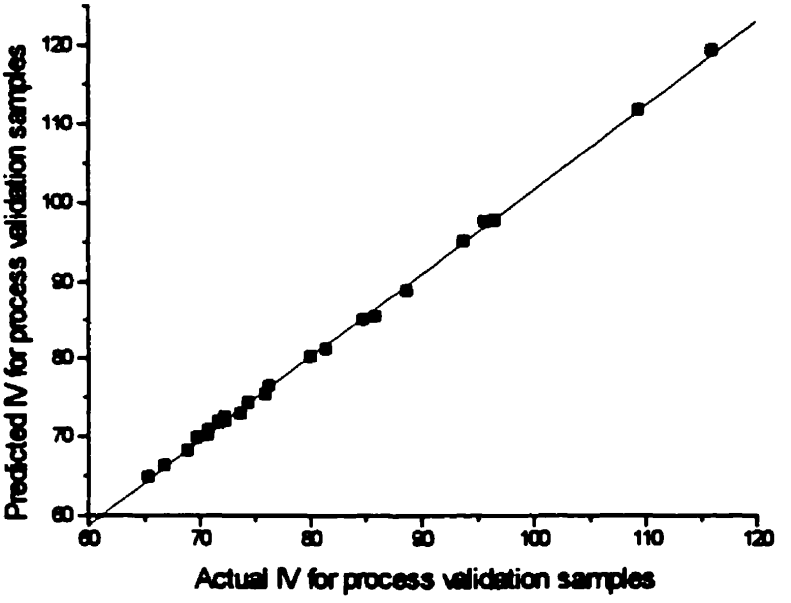

Figure 3.4. Plot of predicted vs. actual IV for the process validation samples. Linear regression equation forced through the origin is y = 1.005x, SD =0.99, r^2=0.999. For abbreviation see Figure 3.3.

Table 3.1. MDa, SDDa and CV for process soybean validation samples relative to the mid-IR reference values[1]

Parameter	n	MDa	SDDa	CV
Cis	23	-0.26	0.96	1.03
Trans	23	-0.02	0.39	0.93
IV	23	-0.28	1.04	0.57

[1]MD, mean difference; SDD, standard deveiation of the differences; CV, coefficient of variation; mid-IR, mid-infrared; n, number of samples; IV, iodine value; a, accuracy

Table 3.2. MDa, SDDa and CV for rapeseed validation samples relative to the mid-IR reference values[1]

Parameter	n	Mda	SDDa	CV
cis	15	-6.53	1.41	2.46
trans	15	0.00	1.89	4.14
IV	15	-4.62	1.50	1.81

[1]For abbreviations see Table 3.1

This relationship was assessed by calculating IV from the FT-NIR *cis* and *trans* predictions using Equation [3.1] and comparing these values to the FT-NIR IV predictions. The relation between the calculated and predicted IV values was:

$$\text{CalcIV} = 1.005\text{PredIV} \qquad \text{SD} = 0.93 \qquad r = 0.999 \qquad [3.2]$$

indicating that the internal consistency expected to exist between the *cis*, *trans* and IV predictions is maintained for the soybean validation set.

As noted earlier, one can expect that a calibration based on a set of process samples will not be generally applicable because the built-in correlations between *cis* and *trans* content are a potential source of predictive error. To evaluate the magnitude of this effect, samples of hydrogenated rapeseed oil were analyzed. Table 3.2 presents the linear regression equations and statistics of the FT-NIR predictions for these samples regressed against their mid-FTIR reference values. As can be seen from the data in Table 3.2, there are definite changes in MD and SDD values relative to those noted in Table 3.1. In particular, the MD values for the *cis* and IV parameters indicate that the FT-NIR rapeseed predictions are significantly biased while the SDD for IV and *trans* is three to four times greater for the rapeseed than for the soybean validation samples. The IV predictions are graphically shown in Figure 3.5 and illustrate the magnitude of the errors which can result when a calibration model with a built-in correlation is applied to samples which do not reflect that correlation.

3.4.3 Generalized Calibration

To minimize the potential errors introduced by developing a calibration based on samples having intercorrelated variables, a calibration set was designed to remove all intercorrelations between the parameters of interest. This set consisted of 29 samples prepared by mixing various oils along with 22 samples of hydrogenated soybean and rapeseed oils. Figure 3.6 presents the mean, variance and correlation (for *cis*, *trans*, IV and SN) spectra for this calibration set. The mean and variance spectra presented in Figure 3.6 appear to be quite similar to the corresponding spectra in Figure 3.3 for the

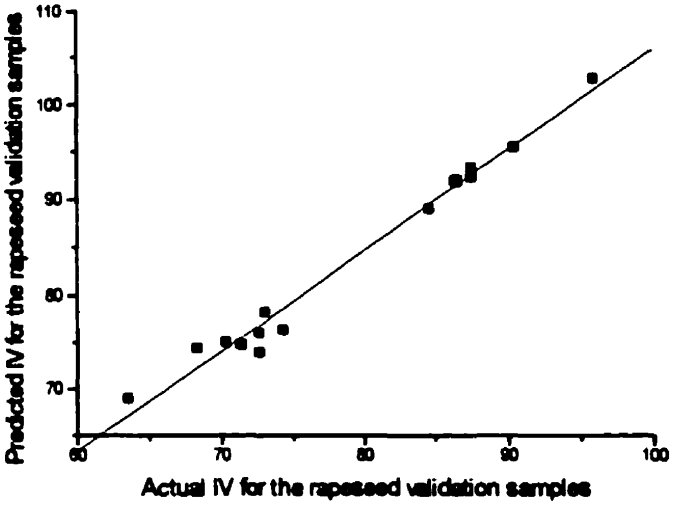

Figure 3.5. Plot of predicted vs. actual IV for the rapeseed validation samples. Linear regression equation is y = -0.291 + 1.062x, SD = 1.43, r^2= 0.991. For abbreviation see Figure 3.3.

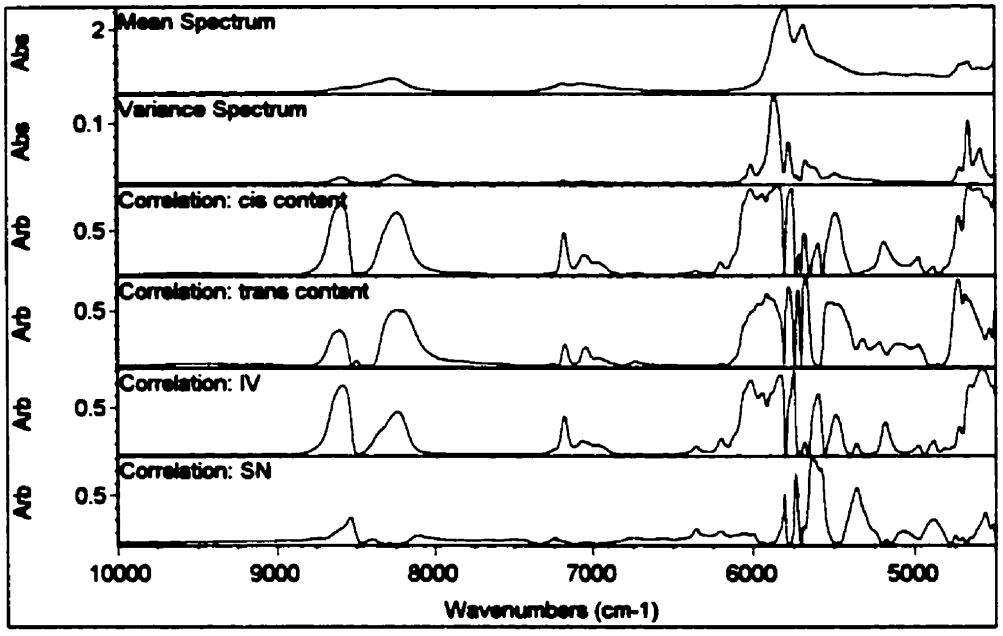

Figure 3.6. Mean, variance and *cis/trans*/IV/SN correlation spectra of the non-correlated calibration standards.

process calibration set. However, Figure 3.6 differs from Figure 3.3 in that substantial differences between the *cis*, *trans*, and IV correlation spectra now exist because there are no intercorrelations between these parameters. As a result, it can be expected that the optimum spectral regions for calibration will be different for each parameter and thus have to be determined independently, making the development of the calibration a more time-consuming process.

Table 3.3 presents the optimal regions chosen for the calibration of each parameter on the basis of the RMSE obtained from a leave-one-out cross-validation, and Table 3.4 presents the cross-validation MD and SDD statistics for accuracy for both the process-based and the generalized calibrations. It can be seen that the process-based calibrations perform somewhat better, likely owing to the supporting intercorrelation of the three parameters measured. In the generalized calibration approach, PLS has to evaluate each of the parameters independently, resulting in some loss of accuracy; however, this approach has the advantage that the calibration devised can readily handle a wide variety of samples, rather than being restricted to samples having particular characteristics. The generalized calibration was validated with a set of 61 samples of hydrogenated soybean and rapeseed oils. A plot of IV predictions vs. the reference IV values for the validation samples being presented in Figure 3.7. Table 3.5, which summarizes the accuracy and reproducibility statistics for the four parameters, shows that the accuracy of the generalized calibrations is largely in line with the leave-one-out cross-validation results (Table 3.4), indicating that *cis*, *trans*, IV and SN can be measured to within 1.3 units of the mid-IR reference values. The reproducibility of the predictions, evaluated using a subset of 20 of the validation samples analyzed two weeks apart, is within one unit for each of the four parameters.

Based on the results obtained in this study, it is apparent that FT-NIR is capable of providing high-quality *cis*, *trans*, IV and SN data from edible oils if the system is properly calibrated. With the use of a heated flow cell accessory a typical analysis takes about two minutes per sample, and analysis can be carried out "at-" or "on-line". The critical issue is of course calibration as FT-NIR is a secondary method of analysis. In this

Table 3.3. Optimized calibration regions for *cis/trans*/IV/SN calibrations

Region (cm^{-1})	Baseline (cm^{-1})	*cis*	*trans*	IV	SN
4698-4553	4825	+[1]		+	
6085-5938	6638	+		+	
8975-7189	7575	+	+	+	
4779-4564	4800		+		
5238-5056	2563		+		
8441-7582	7575				+
8477-8857	7575				+

[1]The plus symbol indicates that the associated spectral region and baseline were used in the calibration model for the parameter. SN, saponification number; for other abbreviation see Table 3.1.

Table 3.4. Cross-validation data for calibrations based on correlated process samples and for generalized calibrations based on non-correlated calibration standards[1]

	Process Based Calibration			Generalised Calibration		
Parameter	n	MDa	SDDa	n	MDa	SDDa
cis	25	0.015	0.75	51	-0.046	1.58
trans	25	0.069	0.49	51	0.009	1.44
IV	25	0.059	0.69	51	-0.070	1.00
SN	25	NA	NA	51	0.001	1.93

[1]NA, not applicable. For other abbreviation see Table 3.1.

Table 3.5. Validation data for the soybean and rapeseed oil samples predicted using the generalized calibrations[1]

Parameter	Mda	SDDa	Na	MDr	SDDr	Nr
cis	-0.346	1.184	60	0.263	0.778	20
trans	-0.272	1.028	60	-0.455	0.451	20
IV	-0.030	0.889	60	0.891	0.686	20
SN	-0.453	1.345	60	0.625	1.074	20

[1]Na, number of samples for which accuracy was evaluated; Nr, number of samples for which reproducibility was evaluated; for other abbreviations see Table 3.1

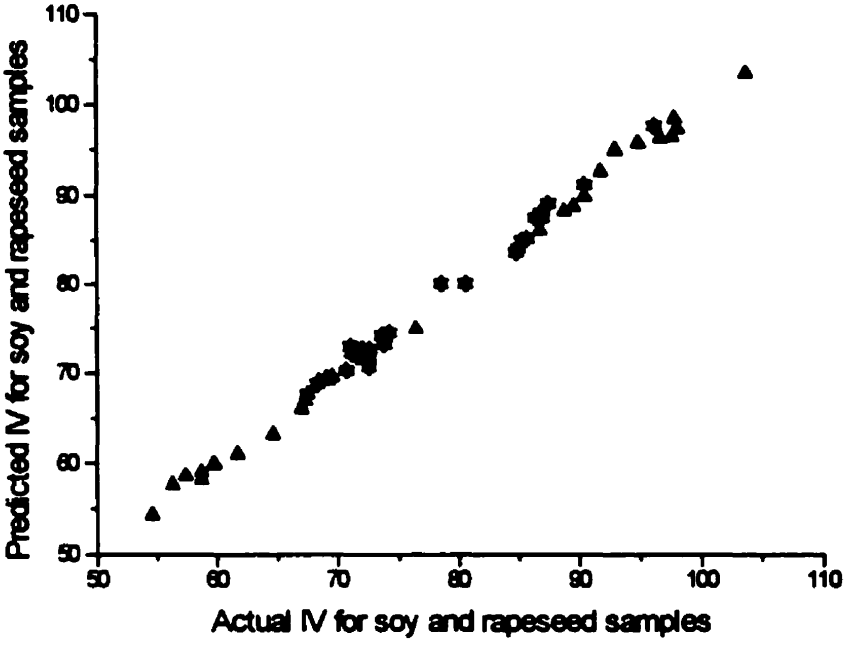

Figure 3.7. Plot of predicted vs. actual IV for the soybean (▲) and rapeseed (▼) validation samples.

study, reference data were conveniently obtained by mid-IR spectroscopy using our EOA Package. In the industrial setting such data would be obtained using standard chemical methods or by gas chromatography. We have demonstrated the inherent limitation associated with the use of process samples as standards for the development of PLS calibrations if the measured parameters are highly correlated. This does not prevent one from calibrating on such samples; however, it is important to ensure that the standards used are representative of the samples to be analyzed and that the limited scope of applicability of the calibrations be recognized. Our preference is to employ a generalized calibration approach and prepare calibration samples, in which the parameters are not correlated, thereby providing the capability to analyze a wide range of oil samples. In conclusion, IV, SN, *cis* and *trans* content of fats and oils can be determined by FT-NIR analysis, providing a more rapid and convenient alternative to conventional chemical and physical methods of analysis.

3.5 Acknowledgements

The authors thank Bomem Inc. and the Natural Sciences and Engineering Research Council of Canada for joint support of this research through an Industrial Partnership Grant. We also gratefully acknowledge Dr. S. Dwight for the sample handling accessory modifications and Dr. P. Maes for providing oil samples used in this work.

3.6 References

1. Haumann B. F., Dietary Fats Still Spark Controversy, *INFORM* 5:358 (1994)

2. van de Voort, F.R., J. Sedman, G. Emo and A.A. Ismail, Rapid and Direct Iodine Value and Saponification Number Determination of Fats and Oils by Attenuated Total Reflectance/Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 69:1118-1123 (1992)

3. Ismail A.A., F.R. van de Voort, J. Sedman, and G. Emo, Rapid Quantitative Determination of Free Fatty Acids in Fats and Oils by FTIR Spectroscopy, *J. Am. Oil Chem. Soc.* 70:335-341 (1993)

4. van de Voort, F.R., A.A. Ismail, J. Sedman, J. Dubois and T. Nicodemo, The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 71:921-926 (1994)

5. van de Voort, F.R., A.A. Ismail, J. Sedman, and G. Emo, Monitoring the Oxidation of Edible Oils by FTIR Spectroscopy, *J. Am. Oil Chem. Soc.* 71:243-253 (1994)

6. van de Voort, F.R., A.A. Ismail, and J. Sedman, A Rapid Determination of *cis* And *trans* Content of Fats and Oils by FTIR Spectroscopy, *J. Am. Oil Chem. Soc.* 72:873-880 (1995)

7. Dunmire, D., and R. Williams, Automated Qualitative and Quantitative NIR Reflectance Analysis, *Cereal Foods World* 35:913 (1990)

8. Dong, J., Kangming Ma, F.R. van de Voort, and A.A. Ismail, Stoichiometric Determination of Hydroperoxides in Oils by Fourier Transform Near-Infrared Spectroscopy, *JAOAC Int.* 80:345-353 (1997)

9. Youden, W.J., and E.H. Steiner, *Statistical Manual of the AOAC*, Association of Official Analytical Chemists, Arlington (1975)

10. Cast J., Infrared Spectroscopy of Lipids, In *Developments in Oils and Fats*, (edited by R.J. Hamilton), Blackie, Glasgow, pp. 224-263 (1995)

11. Sato, T., S. Kawano and M. Iwamoto, Near Infrared Spectral Patterns of Fatty Acid Analysis from Fats and Oils, *J. Am. Oil Chem. Soc.* 68:827-833 (1991)

12. Holman , R. T., and P. R. Edmondson, Near-Infrared Spectra of Fatty Ascids and Some Related Substances, *Anal. Chem.* 28:1533-1538 (1956)

13. Wetzel, D.L., Lipid Structures and NIRA, presented at the 4th International Symposium NIRA, Kansas State University, Manhattan (1983)

14. van de Voort, F. R., FTIR Spectroscopy in Edible Oil Analysis, *INFORM* 5:1038-1042 (1994)

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Chapter 3 demonstrated that PLS calibrations could be derived for FT-NIR using a conventional transmission cell to allow for the simultaneous determination of *cis*, *trans*, IV and SN. In the following chapter the efficiency of a newly devised, simple to use glass-vial sample handling system is evaluated. This evaluation was based on the contention that successful marketing of FT-NIR instrumentation for edible oil analysis, hinged not only on being able to obtain accurate results, but also on ensuring that sample handling was simple and convenient. In particular, Bomem our industrial collaborator suggested the use of cheap, readily available, disposable glass vials. Such vials have inherent dimensional variability and present a curved surface, that are additional constraints on the level of accuracy one might obtain relative to a constant pathlength flow cell. Vials were evaluated through the development of a FT-NIR method for the analysis of low end peroxide values, using as a reference a well characterized and accurate mid-FTIR PV method.

CHAPTER 4 DETERMINATION OF PEROXIDE VALUE BY FOURIER TRANSFORM NEAR-INFRARED SPECTROSCOPY

4.1 Abstract

A Fourier transform-near infrared (FT-NIR) method originally designed to determine the peroxide value (PV) of triacylglycerols at levels of 10-100 PV was improved upon to allow for the analysis of PV between 0 and 10 PV, a range of interest to the edible oil industry. The FT-NIR method uses convenient disposable glass vials for sample handling and PV is determined by spectroscopically measuring the conversion of triphenylphosphine (TPP) to triphenylphosphine oxide (TPPO) when reacted with hydroperoxides. A partial-least-squares (PLS) calibration was developed for 8mm o.d. vials by preparing randomized mixtures of TPP and TPPO in a zero-PV oil. The method was validated with samples prepared by gravimetric dilution of oxidized oil with a zero-PV oil. It was shown that the American Oil Chemists' Society primary reference method was quite reproducible (±0.5 PV), but relatively insensitive to PV differences at lower (0-2) PV. The FT-NIR method on the other hand was shown to be more accurate overall in tracking PV, but slightly less reproducible (±0.9 PV) due to working close to the limit of detection. The sensitivity and reproducibility of the FT-NIR method could be improved upon through the use of larger diameter vials combined with a detector having a wider dynamic range. The proposed FT-NIR PV method is simple to calibrate and implement and can be automated to allow for routine quality control analysis of edible fats and oils.

KEY WORDS: Fourier Transform infrared spectroscopy, FT-IR, FT-NIR, oil analysis, peroxide value

4.2 Introduction

The oxidation of fats and oils is an important deteriorative reaction with significant commercial implications in terms of product value. The initial oxidation products that accumulate in triacylglycerols are hydroperoxides, which may subsequently break down to form lower-molecular weight compounds, such as alcohols, aldehydes, free fatty acids,

and ketones, ultimately leading to rancid product. There are two standard chemical methods (Cd 8b-90 and Cd 8-53) approved by the American Oil Chemists' Society (AOCS) for the determination of hydroperoxides (1). Both are iodometric procedures for determining peroxide value (PV), differing only in the solvent used. Although claimed to be relatively simple, reasonably sensitive, reliable, and reproducible, the iodometric method is labor-intensive and uses significant amounts of reagents and solvents of environmental concern.

The McGill IR Group has worked on the development of methods for the edible oil industry based on Fourier transform infrared (FTIR) and near-infrared (FT-NIR) spectroscopy that allow quantitative analyses to be carried out directly on neat fats and oils, conferring the advantages of analytical speed and automation (2). In terms of PV methodology development, the first FTIR method developed was based on the measurement of the characteristic O-H stretching absorption band of hydroperoxides in the mid-IR (3). Subsequently, a simpler and more accurate mid-FTIR method (4) was developed, based on the well-characterized stoichiometric reaction of triphenylphosphine (TPP) with hydroperoxides to form triphenylphosphine oxide (TPPO). This reaction (Figure 4.1) is rapid and complete when an excess of TPP is present. Accurate quantitation of the TPPO is readily achieved by measuring the intensity of the unique and intense mid-IR absorption band of TPPO at 542 cm^{-1}. The mid-IR method developed was accurate, reproducible and very sensitive, capable of measuring PV down to ~ 0.2 PV (4).

Subsequent work related to monitoring the progress of oxidation in rapeseed lubricants led to the development of other FT-NIR methods for PV determination (5,6). Based on the same concepts as the mid-IR method, the FT-NIR approach provided access to a simpler and more convenient sample handling system, making use of readily available glass vials. Because the objective was to monitor oxidative stress, in a manner analogous to the active oxygen method (AOM), the FT-NIR method was originally devised to measure PV trends over a broad range of PV's (0-100). In this paper, we describe the upgrading of the FT-NIR PV method for the determination of PV over the range of 0-10 PV.

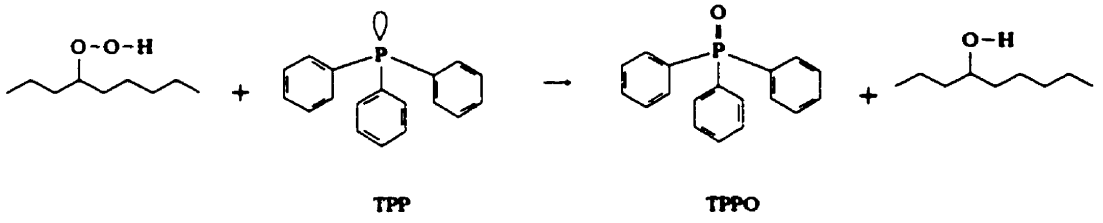

Figure 4.1. Reaction of triphenylphosphine (TPP) with hydroperoxides to form triphenylphosphine oxide (TPPO) and alcohol.

4.3 Materials and Methods

4.3.1 Oil Samples

Canola oil, used as the base oil, was obtained locally. The oil was passed through a column of microwave-activated silica gel to remove partially polar oxygenated molecules, in particular any residual hydroperoxides. The silica gel-treated canola oil was verified to be peroxide-free by the AOCS standard method (Cd 8b-90) (1). Reagent-grade TPP (>99%) and TPPO (>99%) were obtained from Aldrich Chemicals (Milwaukee, WI), and separate concentrated stock solutions (40%, w/w) of TPP and TPPO were prepared in chloroform.

4.3.2 Instrumentation and Sample Handling

The instrument used for this study was a Bomem FT-NIR spectrometer (MB-Series, Bomem Inc., Quebec) equipped with a deuterated triglycine sulfate (DTGS) detector capable of scanning the spectral range of 12,000-2000 cm-1. The spectrometer was controlled by an IBM-compatible Pentium 200-MHz PC running under Windows-based Bomem-Grams/32 software (Galactic Industries Co., Salem, NH). The sample handling accessory used in this study was a temperature-controllable multi-vial holding block (Bomem Inc., Quebec) maintained at 30°C, capable of accepting 8 mm o.d. transparent glass vials (Kimble Glass Inc., Vineland, NJ) which had a volume of ~1 mL. For sample analysis, vials were filled with ~ 0.7-1.0 mL of oil and scanned over the spectral range of 12,000-4000 cm-1. All sample and background spectra were recorded by co-adding 128 scans at a resolution of 4 cm-1. Background spectra were collected every 30 min through an empty vial placed in the vial holder in the IR beam and each sample spectrum was ratioed against the most recently collected background spectrum (Figure 4.2).

4.3.3 Calibration Standards and Validation Samples

Stock solutions (0.5g) of TPP and TPPO (40% in chloroform) were separately incorporated into 100 g of zero-PV canola oil to produce base oils which contained 15 PV

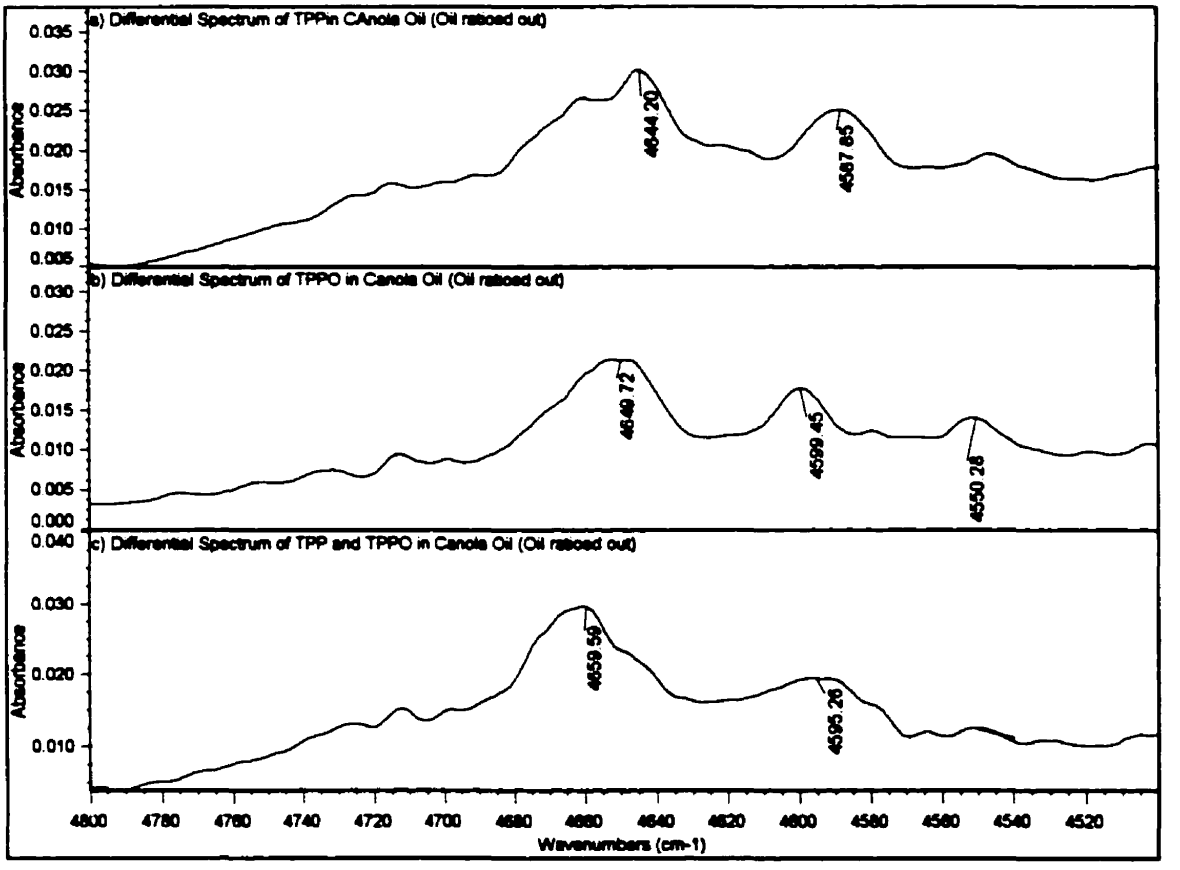

Figure 4.2. Differential spectra of (a) TPP, (b) TPPO, and (c) TPP + TPPO in canola oil produced by ratioing out the spectral contributions of canola oil. See Figure 4.1 for abbreviations.

equivalents of TPP or TPPO, respectively. Calibration standards (~8g) having randomized TPP/TPPO ratios were then prepared by gravimetrically mixing various amounts of zero-PV canola oil with the two base oils (Table 4.1). Randomization was visually verified by plotting the concentrations of TPP and TPPO against each other to ensure that the two-dimensional space of possible combinations of TPP/TPPO concentrations was fairly uniformly spanned (Figure 4.3). Of each standard, ~0.7 mL was transferred to an 8-mm NIR sample vial and scanned as described above. The spectral data was normalized to unit area over the region of 9100-7560 cm-1 and then stored to disk for subsequent development of a partial-least-squares (PLS) calibration model using Omnic TQ Analyst chemometric software (Nicolet Instrument Co., Madison, WI). Correlation and variance spectra were generated to determine where most of the spectral changes in the calibration set took place. These regions were then explored for calibration development and refinement. Each calibration was assessed by using the leave-one-out cross-validation procedure and optimized in terms of the appropriate number of factors by minimizing the predicted residual error sum of squares (PRESS). Validation samples were prepared by gravimetrically mixing oxidized canola oil (PV ~13) and zero-PV canola oil to produce samples having PV values within the range of 0 to 10. Two additional sets of validation samples were prepared in the same manner utilizing olive or sunflower oil, respectively, instead of canola oil.

4.3.4 Sample Analytical Protocol

For sample analysis, the protocol consisted of adding 50μl of 40% stock TPP-chloroform solution with a precalibrated repipette to 15g of the sample, mixing for 20 sec on a Vortex mixer and then transferring *ca.* 0.7 mL to an 8 mm (o.d.) NIR vial. The amount of TPP added was sufficient to react with all the hydroperoxides in an oil having a PV of ~15, well in excess of the measurement range considered (0-10 PV). After scanning the sample, the PV was predicted from the PLS TPP/TPPO calibration. The validation samples were also analyzed in parallel by the AOCS chemical PV method (Cd 86-90) (1).

Table 4.1. Calibration matrix of oil containing TPP and TPPO for developing a partial-least-squares PV calibration[1]

Std. No.	Pure oil (g)	15PV TPP Oil Stock(g)	15 PV TPPO Oil Stock(g)	PV TPP	PV TPPO
1	0	8	0	15.13	0
2	7.1772	0.6184	0.2701	1.16	0.53
3	6.0437	0.706	1.2777	1.33	2.52
4	6.0861	1.2208	0.6934	2.31	1.37
5	5.1475	1.7925	1.2974	3.29	2.49
6	6.5499	0.1468	1.3255	0.28	2.62
7	3.5888	3.4366	1.0816	6.41	2.11
8	5.1104	2.2196	0.6419	4.21	1.27
9	4.7146	1.1785	2.1124	2.23	4.18
10	4.7901	0.6036	2.6548	1.13	5.22
11	3.7153	0.4532	3.9275	0.85	7.68
12	4.3947	2.2084	1.5682	4.09	3.04
13	4.0141	0.86	3.226	1.61	6.3
14	3.209	1.276	3.5474	2.4	6.99
15	3.1966	2.181	2.52	4.18	5.05
16	2.8168	0.6746	4.5317	1.27	8.94
17	2.718	0.1146	5.1514	0.22	10.21
18	2.8407	3.1268	1.9246	5.99	3.86
19	2.9425	4.7076	0.3127	8.94	0.62

[1]PV = peroxide value; the amounts of TPP (molecular weight = 262.28) and TPPO (molecular weight = 278.29) added to zero-PV canola oil are expressed in terms of PV equivalents, based on the stoichiometry of the reaction between TPP and hydroperoxides to form TPPO. Oil containing 1 PV unit of ROOH as determined by the standard iodometric reaction would react with 0.5 mmol TPP/kg oil, producing 0.5 mmol TPPO/kg oil. Hence, 1 PV equivalent of TPP = 0.1311g TPP/kg oil and 1 PV equivalent of TPPO = 0.1391g TPPO/kg oil

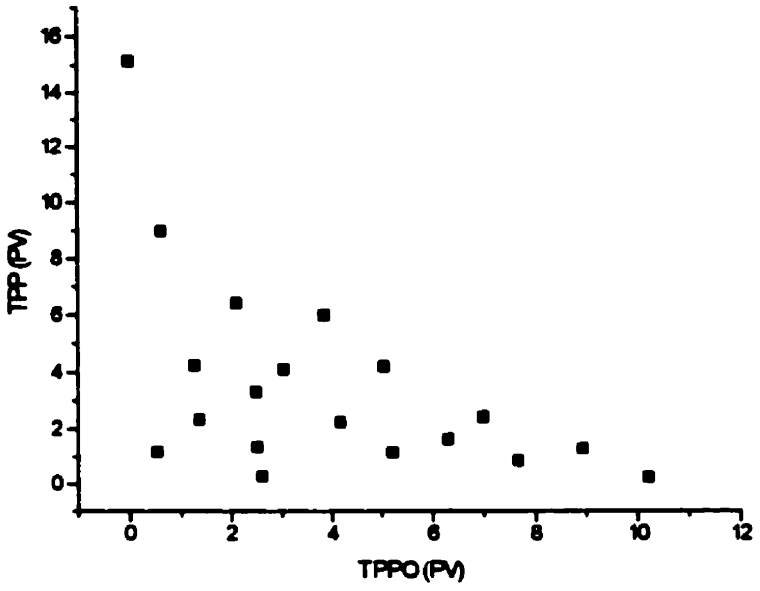

Figure 4.3. A plot illustrating the relative concentrations of TPP and TPPO in the PLS calibration standards. The concentrations of TPP and TPPO are expressed in terms of PV equivalents, in accordance with the stoichiometry of the reaction between TPP and hydroperoxides to form TPPO (1 PV equivalent of TPP = 0.1311g TPP/kg oil and 1 PV equivalent of TPPO = 0.1391g TPP/kg oil). The two dimensional space of possible combinations of TPP/TPPO concentrations is represented by the area below the dashed line. See Figure 4.1 for abbreviations.

4.4 Results and Discussion

4.4.1 General Spectroscopy

The NIR spectral characteristics of TPP and TPPO in canola oil at low concentrations were investigated by adding each component individually to zero-PV canola oil, recording the FT-NIR spectra of the spiked oils, and ratioing out the spectral contributions of the canola oil to produce "differential spectra". Figure 4.2 shows the differential spectra of TPP, TPPO, and a mixture of TPP + TPPO between 4800 and 4500 cm^{-1}. TPPO has similar spectral features to TPP, but the major bands are shifted toward shorter wavelengths. When TPP reacts with hydroperoxides in oil to produce TPPO, both TPP and TPPO are simultaneously present in the oil and the bands of these two components will overlap (Figure 4.2c). Quantitation under these circumstances requires the use of more sophisticated chemometric techniques such as PLS regression (7).

4.4.2 PLS Calibration.

Table 4.1 presents the calibration standard matrix used for the PLS calibration. The FT-NIR determination of PV requires that the amount of TPPO formed by the reaction between hydroperoxides and TPP be quantitated in the presence of unreacted TPP, thus the calibration must be completed with standards containing both TPP and TPPO. The concentrations of TPPO and TPP in the standards, expressed in terms of PV equivalents, are plotted against each other in Figure 4.3 to illustrate their lack of correlation. This is in accordance to PLS requiring that the calibration standards contain randomly varying amounts of potentially interfering components in the samples to be analyzed. A PLS calibration model using five factors was developed to predict PV based on the quantitation of TPPO using the region 4695-4553 cm-1 referenced to a single baseline point at 9143 cm-1. Figure 4.4 presents a plot of predicted versus actual TPPO concentration, expressed in PV equivalents, obtained from cross validation of the PLS calibration. The linear regression equation for this plot was $y = 0.390 + 0.904x$, having a correlation coefficient of 0.98 and a SD of ±0.55 PV.

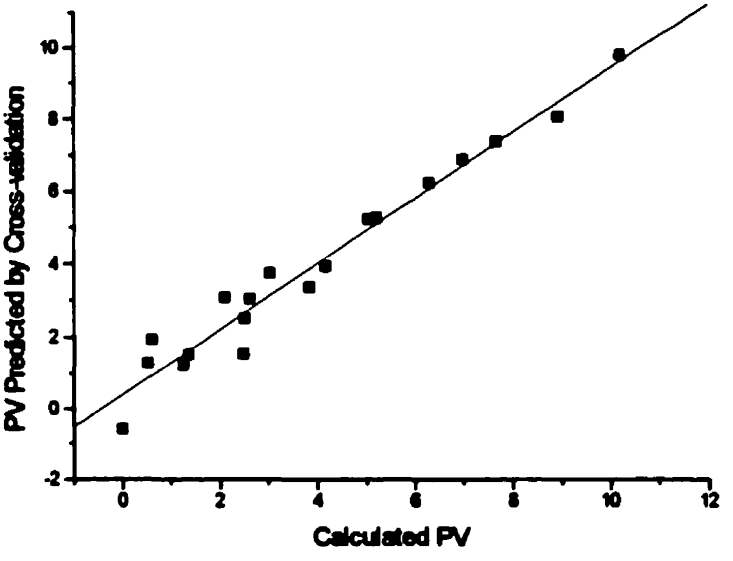

Figure 4.4. A cross validation plot of predicted peroxiide value (PV) vs calculated PV based on the gravimetric addition of TPPO. See Figure 4.1 for abbreviations.

4.4.3 Validation

The PLS calibration model was validated by analyzing a series of samples prepared by gravimetric dilution of an oxidized canola oil with a zero-PV canola oil. Duplicate analyses were performed two days apart and the mean difference for reproducibility (MDr) and the standard deviation of the differences (SDDr) for the NIR duplicate predictions were 0.27 PV and *ca.* ±0.90 PV units, respectively. These validation samples as well as the oxidized oil used to prepare them were also chemically analyzed by the AOCS PV method (Cd 86-90) (1). In addition, the PV of the validation samples were calculated from the chemical PV of the base oxidized oil and the gravimetric dilution factors. Figure 4.5 illustrates the validation plot obtained by plotting the FT-NIR-predicted PV for the validation samples versus their calculated PV, the linear regression equation for this plot being $y = 0.036 + 0.875x$, with a correlation coefficient of 0.964 and an SD of ± 0.95 PV. The mean difference for accuracy (MD_a) and the standard deviation of the differences (SDD_a) for the NIR duplicate predictions were −1.0 PV and *ca.* ±0.67 PV units, respectively. A plot of FT-NIR-predicted PV vs. actual chemical PV (Figure 4.6) indicates some curvature, its linear regression equation being $y = 1.672 + 0.861x$, having a correlation coefficient of 0.93 and a SD of 1.35 with MD_a and SDD_a being -0.95 and ± 1.41 PV, respectively.

A careful assessment of duplicate analyses by the AOCS chemical method indicated that the reproducibility of the chemical method was very good, producing a mean difference for reproducibility (MD_r) of 0.21 PV and a SDD_r of ±0.54 PV. A plot of the duplicates against each other (Figure 4.7) was linear ($r = 0.994$), had a slope close to 1 (1.09), with an intercept close to zero (–0.26 PV) and a SD of ±0.44 PV. These data for the chemical method clearly indicate that one can routinely reproduce PV results to within *ca.* ± 0.50, well within the general expectation of the method of ±1.0 PV. However, a plot of the individual chemical PV results versus calculated PV for the gravimetrically diluted oxidized canola oil samples (Figure 4.8) reveals that the relationship is curvilinear, fitting a quadratic relationship quite well and on this basis, having an SD of *ca.* ±0.72 PV. These results indicate that the chemical PV method, although quite reproducible, does not

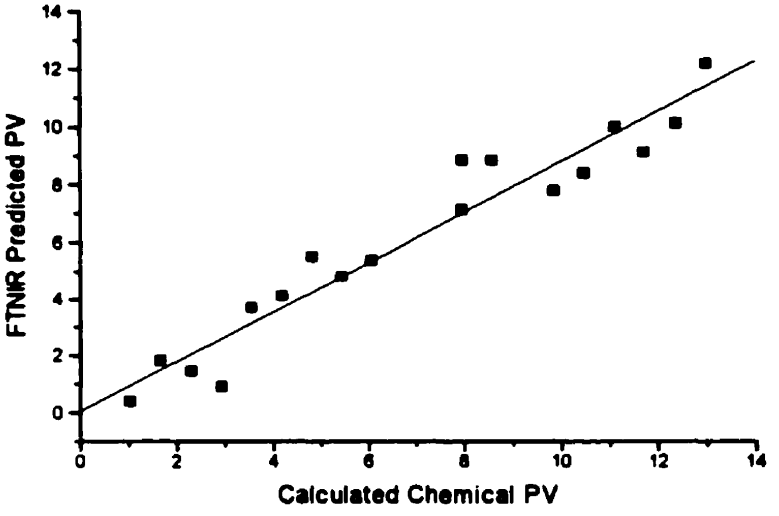

Figure 4.5. Plot of FT-NIR-predicted PV vs. calculated PV for gravimetrically diluted oxidized canola oil samples. See Figure 4.4 for other abbreviations.

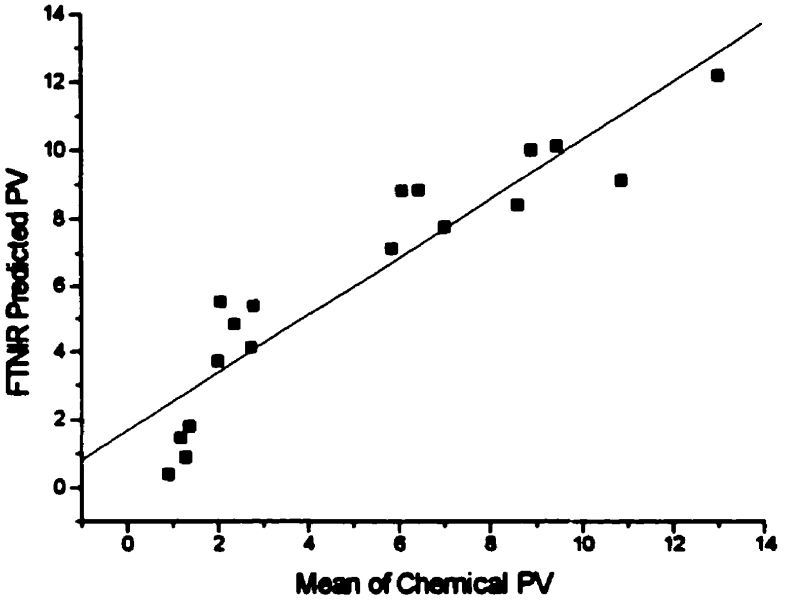

Figure 4.6. Plot of FT-NIR-predicted PV vs. chemical PV for gravimetrically diluted oxidized canola oil samples. See Figure 4.1 and 4.4 for abbreviations.

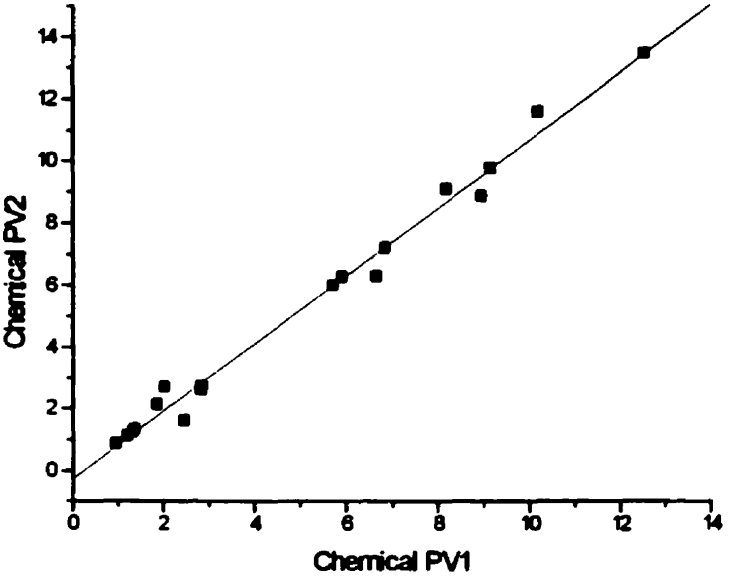

Figure 4.7. Plot of PV data obtained from duplicate chemical analyses of the gravimetrically diluted oxidized canola oil samples. See Figure 4.4 for abbreviations.

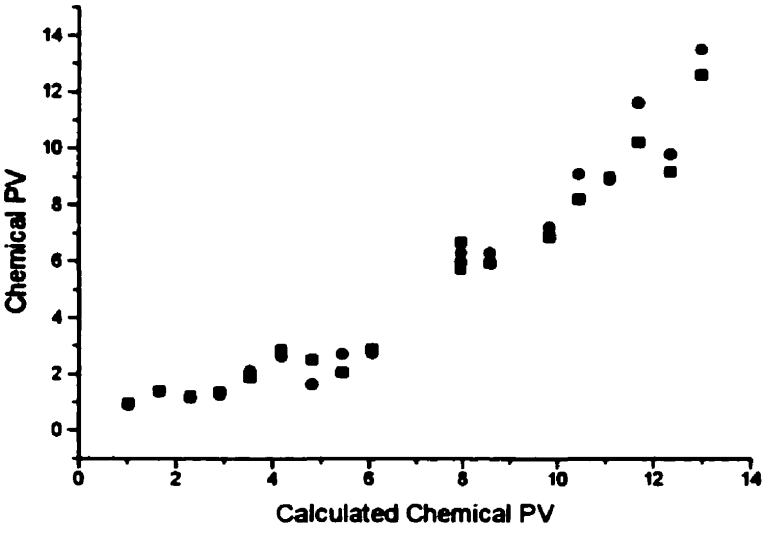

Figure 4.8. Plot of duplicate (■, •) chemical vs. calculated PV values of gravimetrically diluted oxidized canola oil. See Figure 4.4 for abbreviations.

necessarily respond linearly, being relatively insensitive to changes in PV at lower PV values. Without this more careful analysis, it would have been reasonable to conclude that the nonlinearity between instrumental and chemical results (Figure 4.6) is due to the secondary method rather than the primary reference method, but this is clearly not the case. Based on our experience, the reproducibility and sensitivity of the reference method tends to be the limiting factor.

To investigate the scope of applicability of the PV calibration devised in this work, validation sets prepared with olive and sunflower oil and their PV predicted from the canola oil calibration. Linear regression of the predicted PV against the chemically determined values yielded the following equations for olive and sunflower oils, respectively:

FTNIR-PV = -29.18 + 0.899 PV	r = 0.97	SD = 0.50	[4.1]
FTNIR-PV = 13.13 + 1.123 PV	r = 0.99	SD = 0.38	[4.2]

These results indicate that the calibration tracks the PV changes quite well; however, the values are biased in an oil dependent manner. This effect is likely due to a combination of factors, i.e., the presence of interfering absorptions of the oil in the measurement region as well as due to area normalization attributing any changes in area to a pathlength change whereas these changes may arise from differences in fatty acid composition between oil types. The regression errors above indicate that PV calibrations are transferable between oil types. However, if absolute rather than relative PV values are required, regression equations of the types given above need to be developed. In contrast the mid-IR method previously developed (4) is oil-independent and thus universally applicable. Somewhat better reproducibility was also obtained in the mid-IR method, which uses a fixed pathlength transmission cell. However, the use of disposable vials in the NIR method confers advantages of lower cost and simpler sample handling while still providing satisfactory reproducibility provided that the spectra are normalized to compensate for the variability between and within vial lots. Based on the results obtained

in this study, it is clear that one can readily determine the PV of oil samples by FT-NIR spectroscopy over a PV range of 1-10, with a reproducibility of *ca.* ±1.0 PV.

From the standpoint of analysis, the method is quite straightforward, requiring only the weighing of the sample into the vial, adding a fixed amount of excess TPP, mixing gently and scanning the spectrum of the sample. Weighing can be eliminated if accurate and reproducible repipettes are used and the method standardized. The use of disposable glass vials, being particularly attractive from the standpoint of convenience, is an added benefit. Modern FTIR systems, being programmable, effectively allow one to automate a method by developing a user-friendly interface and building the PLS calibration into the system so that a PV value is presented directly to the user after scanning the sample. An FT-NIR instrument configured and calibrated in the manner described in this paper would be a useful tool for the routine quality control analysis of finished and stored oil products.

4.5 Acknowledgements

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4.6 References

1. American Oil Chemists' Society, *Official and Recommended Practices off the American Oil Chemists' Society*, 4th Ed., American Oil Chemists' Society, Champaign, IL (1989)

2. van de Voort, F.R., J. Sedman and A.A. Ismail. Edible Oil Analysis by FTIR Spectroscopy. *Laboratory Robotics and Automation* 8:205-121 (1996).

3. van de Voort, F. R., A. A. Ismail, J. Sedman, J. Dubois and T. Nicodemo, The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 71:921-926 (1994).

4. Ma, K., F. R. van de Voort, J. Sedman and A. A. Ismail, Stoichiometric Determination of Hydroperoxides in Fats and Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 74:897-906 (1997)

5. Dong, Jun, K. Ma, F. R. van de Voort, and A. A. Ismail, Stoichiometric Determination of Hydroperoxides in Oils by Fourier Transform Near-Infrared Spectroscopy, *J. AOAC Int.* 80:345-352 (1997).

6. Ma, K., F.R. van de Voort, A.A. Ismail and J. Sedman. Quantitative determination of hydroperoxide formation by Fourier transform infrared spectroscopy using a disposable infrared card, *J. Am. Oil Chem. Soc.* 71:1095-1101 (1998).

7. van de Voort, F. R., FTIR spectroscopy in edible oil analysis, *INFORM* 5 (9):1038-1042 (1994)

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Lipton, a US based food company, invited FTIR manufacturers to participate in a collaborative study to demonstrate their ability to deliver IV data using FTIR spectroscopy. This required correctly classifying the oil samples among four types and accurately predicting IV values for a series of "unknown" oils, provided by Lipton, who had determined the IV data by standard AOCS methods. The McGill IR Group participated in this collaborating study on behalf of Bomem and developed product-specific IV calibrations for the oils provided by Lipton and also used the "unknown" oils to evaluate a "Global" IV calibration developed by Bomem. The Lipton collaborative study was used to validate the performance of the vial sample handling system which had performed well using spectral normalization (Chapter 4) and also provided an opportunity to explore the discriminant abilities of PLS.

CHAPTER 5 DISCRIMINATION OF EDIBLE OIL PRODUCTS AND QUANTITATIVE DETERMINATION OF THEIR IODINE VALUE BY FOURIER TRANSFORM NEAR-INFRARED SPECTROSCOPY

5.1 Abstract

This work demonstrates the application of partial least squares (PLS) analysis as a discriminant as well as a quantitative tool in the analysis of edible fats and oils by Fourier transform near-infrared (FT-NIR) spectroscopy. Edible fats and oils provided by a processor were used to calibrate a FT-NIR spectrometer to both discriminate between four oil formulations as well as determine the iodine value (IV). Samples were premelted and analyzed in glass vials maintained at 75°C to ensure that the samples remained liquid. PLS calibrations for the prediction of IV were derived for each oil type by using a subset of the samples provided as the PLS training set. For each oil formulation (type), discrimination criteria were established based on the IV range, spectral residual and PLS factor scores output from the PLS calibration model. It was found that all four oil types could be unambiguously differentiated from each other and all the validation samples, including a set of blind validation samples provided by the processor, were correctly classified. The PLS-predicted IVs for the validation samples were in good agreement with the gas chromatography IV values provided by the processor. Comparable predictive accuracy was obtained from a calibration derived by combining samples of all four oil types in the training set as well as a global IV calibration supplied by the instrument manufacturer. The results of this study demonstrate that by combining the rapid and convenient analytical capabilities of FT-NIR spectroscopy with the discriminant and predictive power of PLS, one can both identify oil type as well as predict IV with a high degree of confidence. These combined capabilities provide processors better control over their process.

KEY WORDS: Discriminant analysis, fats and oils, Fourier transform near-infrared spectroscopy, iodine value, quality control, partial least squares, quality control.

5.2 Introduction

The application of near-infrared (NIR) spectroscopy in edible oil analysis has predominantly involved its use for the rapid quantitative determination of the oil content in oilseeds, with relatively little work being carried out on the analysis of oils *per se*. Most work on NIR oil analysis development has focused on classifying and/or discriminating between oil types as well as detecting adulteration, particularly of olive oil. Bewig *et al.* (1) used a filter-based NIR instrument to differentiate between four types of oils (cottonseed, canola, soybean, and peanut) using discriminant analysis based on Mahalanobis distance principles. Sato (2) used principal-component analysis (PCA) to classify vegetable oils using second-derivative NIR spectra, PCA providing the benefit of using all the spectral data collected rather than only the data at selected wavelengths. Wesley *et al.* (3,4) worked on olive oil adulteration and demonstrated that it is possible to effectively use NIR spectroscopy in conjunction with PCA to predict both the purity of olive oil and the type of adulterant as well as to quantitate the adulterant using partial-least-squares (PLS) regression. These studies indicate that discriminant analysis, PCA, and PLS are potentially powerful tools for qualitatively characterizing oils as well as detecting adulteration and estimating the levels of adulterants.

The McGill IR Group has focused on the development of Fourier transform infrared (FTIR) spectroscopic methods for the rapid quantitative analysis of edible oils, based on measurements in the mid-IR region of the spectrum (5-9). Recently, two FT-NIR oil analysis methods have been developed, one for the determination of peroxide value (PV) and the other for the simultaneous determination of *cis* content, *trans* content, iodine value (IV), and saponification number (SN) of edible oils (10,11). This work has elicited positive feedback from industry sources, citing the ease of sample handling and amenability to at-line and on-line implementation as important attributes of FT-NIR oil analysis methods. For NIR oil analysis, disposable glass vials are simply filled with neat sample and discarded after measurement. Thus, NIR analysis is more suitable for industrial applications than mid-IR analysis, which employs transmission IR cells with narrow path lengths (typically 0.025 mm) and salt windows. NIR analysis can also be performed remotely with the use of low-cost NIR-transmitting fiber optics. Conventional

dispersive NIR instruments can be unreliable in terms of maintaining calibration stability, but this problem has been largely overcome with a new generation of FT-NIR instruments which have superior wavelength reproducibility and stability, thereby minimizing calibration drift and reducing the need to recalibrate.

In recent years, several groups have used NIR spectroscopy to characterize and classify different types of fats and oils. This work has largely been based on the application of qualitative analysis techniques such as discriminant analysis based on measurements at selected wavelengths and PCA. However, quantitative analysis techniques such as PLS can also serve as a basis for classification through the setting of discrimination criteria based on the output obtained from the calibration models. There is substantial interest in the edible oils sector in instrumental models for both rapid identification of the type of oil and the quantitative of specific oil parameters such as IV and *trans* content as well as discriminating between oils. This work reports the protocol and results of an investigation of the capabilities of FT-NIR spectroscopy as a practical at-line process control tool for discriminating between various formulated oil products as well as determining their IV.

5.3 Materials and Methods

5.3.1 Oil Samples

For this work, four sets of fats and oils (A-D) were sent to the McGill IR Group by an oil processor. These samples had all been preanalyzed for IV by gas chromatography (GC), and the GC-IV data were provided with the samples. The IV of the samples ranged from 133.3-134.8 for oil A, 91.3-96.3 for oil B, 117.1-118.8 for oil C, and 113.7-117.0 for oil D. In addition, 35 unknowns also were provided as a blind validation set to be used in evaluating the accuracy of product classification and IV determination by FT-NIR spectroscopy. The FT-NIR prediction results obtained for these samples were subsequently sent to the processor, who then made the classification and GC IV data for these samples available for statistical analysis.

5.3.2 Instrumentation and Sample Handling

The instrument used in this study was a Bomem FT-NIR analyzer (Bomem Inc., Quebec) equipped with a deuterated triglycine sulfate (DTGS) detector capable of scanning the spectral range of 12,000-2000 cm^{-1}. The spectrometer was controlled by an IBM-compatible 486 DX-66 MHz PC running under Windows-based Bomem-Grams/386 software (Galactic Industries Co., Salem, NH) and AIRS, a specific quality assurance program produced by DHC Analysis (Cleveland, OH). The sample handling accessory used in this study was a temperature-controllable multivial-holding block capable of accepting 8-mm (o.d.) transparent glass vials (Kimble Glass Inc., Vineland, NJ), having a volume of ~1 mL. Figure 5.1 illustrates the sample handling accessory installed in the spectrometer. The temperature of the sample handling accessory was held at 75°C (± 0.2°C). For sample analysis, vials were filled with 0.5-0.7 mL of oil or premelted fat and scanned over the range of 12,000-4500 cm^{-1}.

All sample and background spectra were recorded by co-adding 128 scans at a resolution of 16 cm^{-1}. Air background spectra were collected every 30 min with the vial holder in the IR beam, and sample spectra were ratioed against the most recently collected air background. The ratioed spectra were subsequently normalized to account for inherent variations in the vial pathlength by using a normalization routine in the AIRS software.

5.3.3 Standards, Software, and Calibration

From each oil product category provided, 20 samples were used as calibration standards and 5 samples were employed as validation samples. The calibration standards were scanned twice and the glass vial containing the sample was rotated 90° in the vial holder between the two scans. The validation standards were run in duplicate. The spectra of the calibration standards together with the GC IV data obtained from the processor were combined in the PLSPlus chemometrics program (Galactic Industries Co., Salem, NH) to develop PLS-NIR IV calibrations. The predicted residual error sum of squares (PRESS) test as well as the root mean square deviation (RMSD) associated with the cross validation of the calibrations tested were used to select optimal calibrations. The

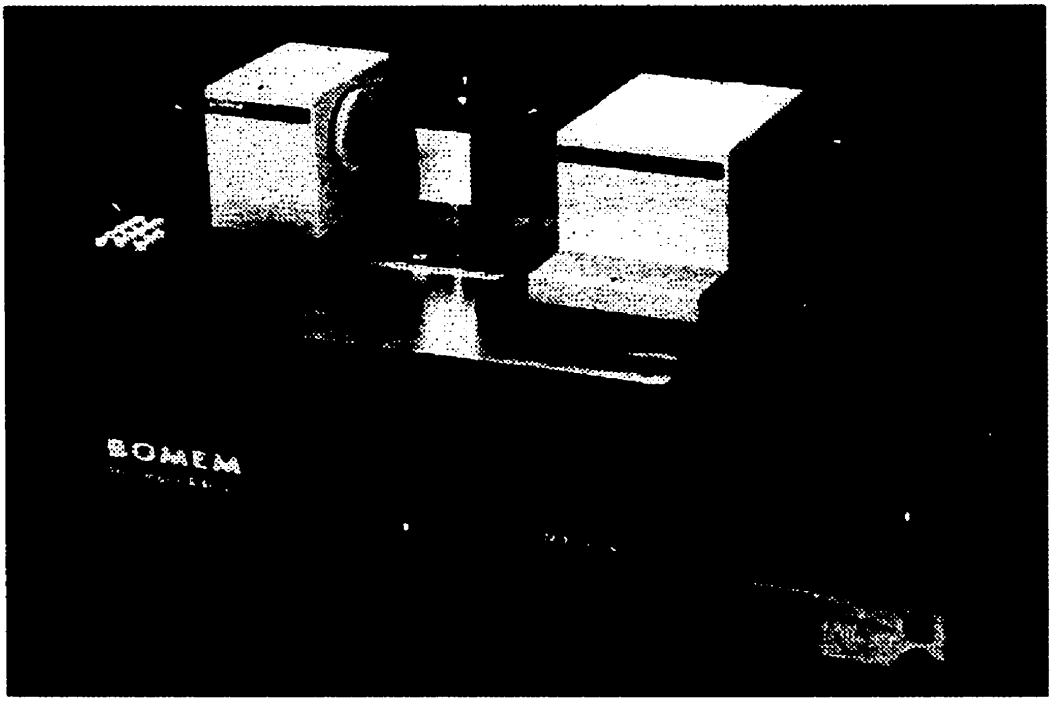

Figure 5.1. The Bomem (Quebec, Canada) MB series Fourier transform near-infrared (FT-NIR) spectrometer with temperature-controlled multivial-holding block.

performance of the calibrations was evaluated by linear regression of the cross-validation predictions and the predictions for the validation samples against the GC data provided by the oil processor, with uncertainty and reproducibility assessed using mean differences (MD) and standard deviation of the differences (SDD) according to the method of Youden and Steiner (12). In addition to the PLS calibrations developed for IV using the samples provided, a global IV calibration provided by Bomem was also assessed. This calibration was developed using a broad selection of edible oils and fats that were collected from over 15 facilities worldwide (13). These oils and fats varied in type and degree of hydrogenation. Oil classification was carried out using AIRS, which provides a means by which to discriminate between sample types or categories based on (i) the PLS-predicted value of a parameter being measured; (ii) the spectral residual, representing the spectral data not fit by the PLS calibration model; or (iii) the PLS factor scores associated with the spectrum, or any combination of the three, as explained below.

5.3.4 Concepts and Principles of the FT-NIR PLS Method

PLS is a powerful multivariate analysis technique that has largely been pioneered for NIR applications and has played a major role in the recent resurgence of quantitative mid-IR spectroscopy. PLS develops a calibration model by compressing the spectral data for a set of calibration standards into a series of orthonormal basis vectors, known as loading spectra or factors. The basis vectors selected to model the spectra of the calibration standards emphasize the spectral variations due to differences in concentration and do not model variations due to random noise, hence the name partial least squares. A PLS calibration can, in principle, be based on the whole spectrum, although in practice the analysis tends to be restricted to regions of the spectrum that exhibit the strongest variations with changes in the concentrations of the components of interest. PLS decomposes the spectrum of each calibration standard into a weighted sum of the loading spectra, and the weights given to each loading spectrum, known as "scores", are regressed against the concentration data for the standards. When the spectrum of an unknown is analyzed, PLS reconstructs the spectrum from the loading spectra. The amounts of each loading spectrum employed in reconstructing the spectrum, i.e., the "scores", are then

used to predict the concentration of the unknown. PLS also generates a spectral residual, which corresponds to the difference between the actual and the reconstructed spectrum.

The scores and spectral residual provide useful information for the detection of outliers, i.e., samples whose spectra differ significantly from those of the training set. Outlier detection is valuable in relation to assessing quantitative accuracy in the prediction of unknowns since, if an unknown is an outlier, the PLS-predicted value for the parameter of interest cannot be considered reliable. It also provides a means of classifying an unknown as being part of the population modeled by the training set, likely being part of this population or not being part of this population on the basis of selected criteria. These criteria may be based on the value of the parameter being predicted, the magnitude of the spectral residual, and the factor scores determined for the unknown. The underlying basis for each of these criteria is as follows: (i) Value within range. If an unknown is part of the population represented by the training set, then the value of the predicted parameter should be within the range of values spanned by the training set. (ii) Residual. When an unknown is predicted, a residual spectrum is computed by subtracting the actual spectrum of the sample from the synthetic spectrum generated by PLS. In order to obtain a numerical value for the magnitude of the residual, the absorbance values at each data point of the residual spectrum are squared and then summed, and then the square root of the sum is taken. If an unknown is part of the population represented by the training set, then the value of the spectral residual should not exceed the average residual encountered in the training set by a large factor. The lower limit for the spectral residual is necessarily set to zero because a zero residual would represent a perfect fit of the spectral data for the unknown. (iii) Factor scores. When a sample is analyzed using PLS, the output includes the contribution (factor score) of each loading spectrum or factor to the synthetic spectrum generated by PLS. When the factor scores for an unknown are within the range of the scores obtained for the training set, it is a very strong indication that the sample truly belongs to the population modeled by the training set. Thus, the criterion based on factor scores is probably the most important classification tool of the three.

The concepts, up to this point, consider only a single population, but can be expanded to consider several sample populations simultaneously. In our work, four oil types (populations) were considered with the objective being to classify unknowns as belonging to one of these types as well as to determine IV. In principle, provided the oil types are sufficiently distinct from each other from a spectral standpoint, this can be achieved by developing a PLS calibration for the prediction of IV for each oil type and then classifying the unknowns on the basis of the output from PLS. This type of classification can be implemented through the AIRS software package, which allows one to develop a method to carry out the following sequence of events: (i) When a sample is analyzed by AIRS, the IV will be predicted from each of the PLS calibration models developed for the different oil types and the discrimination criteria selected for each model (based on range, residual and factor scores) will be applied. (ii) Provided that the sample belongs to one of the oil types and that there is sufficient difference between the oil types, the sample analyzed will pass the discrimination criteria for only one of the models and will accordingly be classified as belonging to the corresponding oil type. (iii) The IV prediction from the selected calibration model will then be reported.

The creation of a method of this type by the AIRS software extends PLS beyond its basic predictive capabilities, providing a basis for classification of a sample within a defined class or among defined classes of oil types. This ability can be particularly useful in quality control situations where one may want not only to predict the value of a parameter but also to confirm to which oil type the sample belongs.

5.4 Results and Discussion

The concepts and principles discussed above have been applied in the development of a FT-NIR method for the discrimination among four defined oil types provided by a processor. PLS calibrations for the prediction of IV from the FT-NIR spectra in the region between 9100 and 7560 cm^{-1} were first individually developed for each oil type using 20 oil samples of that type as the training set. This spectral region was chosen as it contains on-scale overtone bands containing information related to the degree of unsaturation.

Table 5.1 presents the mean, SD, and range of the GC IV data of the standards used for the development of the PLS calibrations, as well as the cross-validation statistics obtained from those calibrations in terms of mean difference (MD_a) and standard deviation of the differences (SDD_a). The MD_a and SDD_a reflect, respectively, the bias and the random error of the PLS predictions. The cross-validation statistics indicate that the IV theoretically can be predicted to within a standard deviation of 0.20 IV units or less for oils A, C and D, whereas for oil B, the SDD_a is ~0.50.

Figure 5.2 presents a composite plot of the individual cross-validation predictions for each of the oil types, showing each oil grouping in a tight cluster except for oil B, which appears to be composed of two subgroups. This somewhat broader spread for oil B may explain the substantially higher SDD_a obtained for this oil. In relation to the feasibility of discriminating between the four oil types on the basis of IV only, oils C and D could potentially pose problems. Although fairly similar in their IV values, careful inspection of their respective SD and ranges indicates that their IV values in fact have no overlap. On the other hand, on the basis of the PLS cross-validation predictions, the situation is less clear-cut in terms of discriminating between oils C and D on the basis of predicted IV alone because the SDD_a values for both these oils exceed the difference between their ranges. Therefore, a more elaborate discrimination strategy was employed.

Three discrimination criteria based on predicted IV, the spectral residual, and the factor scores were used for classification of samples among the four oil types. In the case of factor scores, the score ranges for only the first two factors in the calibration model were found to be sufficient for discrimination between the four oil types. Figure 5.3 presents a schematic representation of the three discrimination criteria set up in the AIRS program to determine whether a validation sample belongs to oil type A. The diagram indicates how the program renders its decisions as to whether a sample belongs, likely belongs or does not belong to the oil type A population using the three criteria. As shown, each criterion is defined by two sets of limits. The inner region I represents the region where a sample is considered to be part of the population, while samples falling in regions II are likely part of the population, and samples falling into regions III are definitely not part of

Table 5.1. GC IV data and PLS cross-validation statistics for oil types A-D[1]

Oil	Mean GC-IV	SD GC-IV	Range GC-IV	PLS-MD_e	PLS-SDD_e	N
Oil A	134.01	0.39	133.3-134.8	0.0005	0.2164	20
Oil B	93.90	1.79	91.3-96.2	0.0001	0.4827	20
Oil C	117.85	0.51	117.1-118.8	0.0020	0.1940	20
Oil D	115.36	1.11	113.7-117.0	-0.0047	0.1468	20

[1]GC, gas chromatography; IV, iodine value; PLS, partial least squares; SD, standard deviation; SDD_e, standard deviation of differences; MD_e, mean difference.

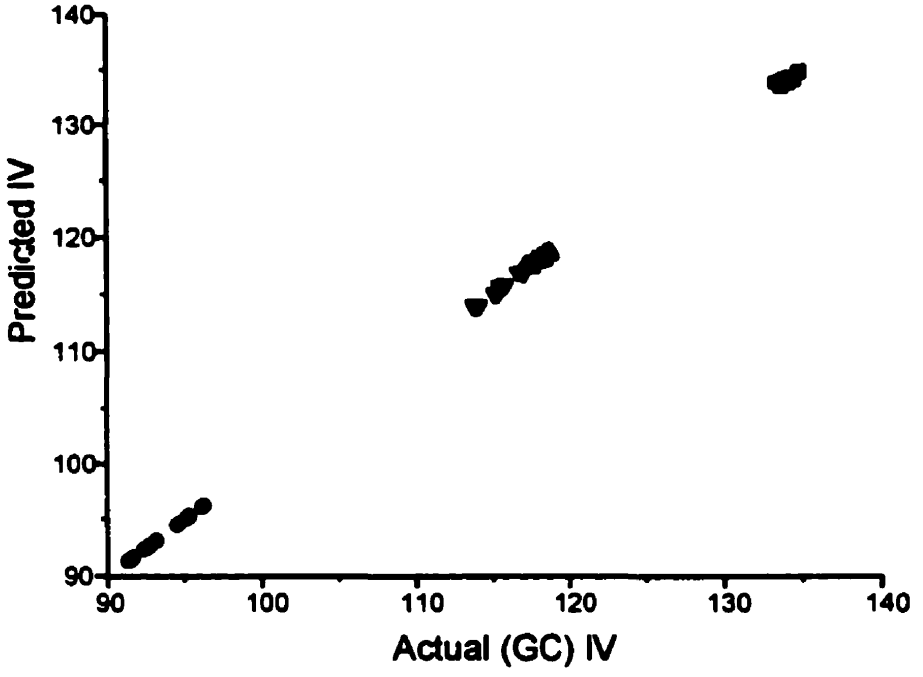

Figure 5.2. Composite plot of the cross-validation results for the four calibrations, showing the predicted iodine value (IV) for the individual training sets vs. the gas chromatography (GC) IV data. ■ Oil A, • oil B, ▲ oil C ▼ oil D.

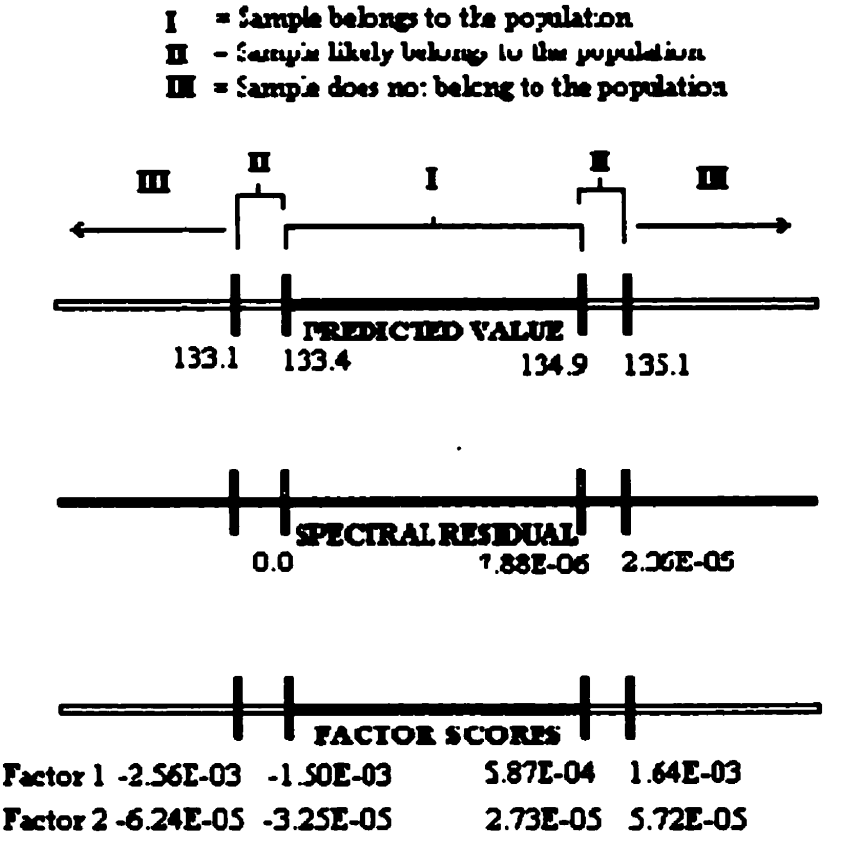

Figure 5.3. Schematic representation of the partial least squares (PLS) based discrimination criteria (predicted IV, spectral residual and factor scores) used to accept (region I), accept with qualification (regions II) and reject (regions III) the oil samples as belonging to oil type A. See Figure 5.2 for abbreviation.

the population. As noted earlier, region I is rigorously defined on the basis of PLS results obtained for the training set, and the limits of this region should in theory be the basis for acceptance or rejection of a sample as belonging to a population. The calculated rate of incorrect classification (i.e., a false positive) based on these rigorously defined discrimination criteria is frequently less than 1 part in 1 million. It is much more likely, however, that a sample belonging to a certain population will not be classified as such (i.e., a false negative), owing to minor deviations in the spectra, and thus a degree of tolerance is built into the discrimination criteria based on the variability of the spectra in the training set. Hence, the additional set of limits (regions II) is included for each discrimination criterion, which can be adjusted to attain a degree of discrimination commensurate with the characteristics of the samples one is working with. In our work, the limits defining regions II were set as follows: range, limits of region I ± root mean square error obtained from cross validation of the calibration; residual, lower limit is zero by default; upper limit is twice the upper limit of region I; and factor scores, limits of region I ± half the range spanned by region I.

Table 5.2 presents the classifications obtained for 20 validation samples, five from each oil type, using the three discrimination criteria based on these limits as well as each discrimination criterion individually. For the majority of the samples, the use of the inner region I yields the correct classification with each of the individual discrimination criteria. The results for oil A illustrate the need to build some tolerance into the discrimination criteria. Because the value of the spectral residual from the PLS calibration for oil A is very small, three of the five validation samples belonging to this oil type would be rejected on the basis of the spectral residual discrimination criterion with the use of region I only. However, when the spectral residual discrimination criterion is extended to include regions II, the sample identification is inconclusive in several cases, indicating the need to base the classification on more than a single discrimination criterion. The results from the classification when the three discrimination criteria were combined in the AIRS program were 100% correct; the samples marked with an asterisk in Table 5.2 were flagged by the AIRS program as possible outliers without any indication of which criteria were marginal.

Table 5.2. Classification of 20 validation samples among oil types A-D using PLS-based discrimination criteria

Sample code	Actual (GC) IV	Range, Residual, and Factor Scores[1, 2]	Range Only[1]	Residual Only[1]	Factor Scores Only[1, 2]
A1073	134.266	A*	A	A*, B*	A
A1078	134.506	A	A	A, B*	A
A1083	133.993	A*	A	A*	A
A1088	134.238	A	A	A, B*	A
A1093	133.927	A*	A	A*	A
B1566	95.2383	B	B	B	B
B1571	94.3976	B	B	B	B
B1576	94.2613	B	B	B	B
B1581	94.6988	B	B	B	B
B1586	94.8791	B*	B	B	B*
C2216	118.76	C*	C	C, D*, B*	C*
C2221	118.01	C	C	C, D*, B*	C
C2226	118.528	C*	C*	C, D*, B*	C
C2231	118.293	C	C	C*, D*, B*	C
C2236	118.133	C	C	C, D*, B*	C
D2556	115.378	D	D	D, C*, B*	D, C*
D2561	115.233	D	D	D, C*, B*	D
D2566	115.481	D	D	D, C*, B*	D
D2571	115.173	D	D	D, C*, B*	D
D2576	115.108	D	D	D, C*, B*	D

[1]Classification of samples without an asterisk is based on region I, those with an asterisk on regions II.
[2]Factor scores were those for the first two factors in the calibration model. See Table 5.1 for abbreviations.

For circumstances in which classification is not of interest *per se*, we investigated the predictive performance of a calibration developed for the four oil types combined together rather than calibrating for each oil type individually. In addition, we evaluated the relative predictive accuracy of a "global calibration" developed and supplied by Bomem (13). Table 5.3 presents the MD and SDD for uncertainty and reproducibility for the pooled data for the validation samples. As can be seen, the individual and combined calibrations perform comparably, while the global calibration has a small bias (< 1.0 IV unit) and a somewhat larger error associated with it. The global calibration, which was developed using an extensive selection of oil types (13), actually performs very well in tracking IV, but requires a bias adjustment in order to match the agreement obtained with the calibrations developed for the specific oil types. As seen from the data in Table 5.3, it does not matter whether one calibrates on oil types individually or together, except that the capability for classification is clearly lost in the latter case. However, the combined calibration has the advantage that it can be applied over a wider IV range with the possibility of interpolation for blends of the different types of oils, which have IV values outside the ranges of the individual oil types.

Although additional information was not provided by the processor about the samples supplied for evaluation in this study, one can surmise, based on the IV data provided, that oils C and D are two different blends of oils A and B. If this were the case, quality control would be interested in knowing which oil or blend is being processed and verifying that the IV is within specifications. By using the individual calibrations, one is capable of both classifying a sample as one of the four oil types and obtaining an accurate value for its IV. If a formulation error has been made (e.g., blending oils A and B in the wrong ratio), the sample would be rejected as an outlier by all four individual calibrations and no IV prediction would be obtained. In this situation, having a combined calibration would be useful. On the basis of the discrimination criteria for the combined calibration, it would be possible to ascertain whether the sample is in fact some blend of oils A and B and, if it is, to obtain a prediction for its IV. Taking this a step further, if the sample were completely unrelated to oils A and B and their blends, the combined calibration would reject the sample and, by resorting to the global calibration, the IV value could be

Table 5.3. Mean difference and standard deviation of the differences of the IV predictions for 20 validation samples from the individual, combined, and global calibrations relative to the GC IV reference values

Statistic[1]	Individual calibrations	Combined calibration	Global calibration
MD_a	0.15	0.02	0.89
SDD_a	0.32	0.33	0.49
MD_r	0.01	0.03	-0.08
SDD_r	0.15	0.15	0.34

[1] a = uncertainty, r = reproducibility. See Table 5.1 for other abbreviations.

predicted, possibly providing a clue as to what type of oil the sample may be. The scenario described above illustrates how the AIRS program would allow one to analyze and troubleshoot a sample by FT-NIR spectroscopy and make practical use of a PLS-based classification method in a quality control situation.

5.4.1 Analysis of Unknowns

Although the results presented in Table 5.3 were derived using proper validation techniques and samples, an additional blind validation study was conducted to provide an added degree of confidence in the method. Table 5.4 presents the results obtained for classification of the blind unknowns as well as their IV predictions from the individual, combined, and global calibrations. This table also includes the information provided to us after completion of the validation study by the processor supplying these samples. Twenty of the 35 samples were identified by the processor as belonging to oil types A-D, and all but one of these had been correctly classified by the FT-NIR method. Approximately half of these correct classifications were based on the less rigorously defined discrimination criteria (regions II), again illustrating the need to build some tolerance into the discrimination criteria. It is particularly noteworthy that despite the similarity of oil types C and D in terms of their IVs, the FT-NIR method was successful in distinguishing between these two oil types.

Five unknowns were incorrectly blended samples of oil type B that had been deliberately included in the validation set to test the capability of the FT-NIR method to detect formulation errors. All five of these samples were rejected by the calibration model for oil type B and only passed the discrimination criteria for the combined calibration model. The IV predictions obtained for these samples from the combined calibration model indicated that the IVs of these samples were about 5 IV units above the IV range for oil type B. This example provides an excellent illustration of the point made previously regarding the utility of the combined calibration model in cases of formulation errors.

The 10 remaining unknowns were identified by the processor as being of types E and F, and thus did not belong to any of the oil types on which the FT-NIR method was

Table 5.4. PLS-based classification and IV predictions for a blind sample set

Code	PLS Classification[1]	PLS-IV Individual	PLS-IV Combination	PLS-IV Global	Actual Classification	Actual IV
U2696b	B	95.05	94.61	94.89	B	95.39
U2701b	B[f]	95.37	95.02	95.34	B	94.87
U2706b	B	94.92	94.77	95.14	B	94.27
U2711b	B	95.22	94.78	95.3	B	94.53
U2716b	A[r]	134.4	134.2	135	A	134.1
U2721b	B[r]	95.76	94.62	95.5	B	94.37
U2726b	A	134.3	134.2	135.1	A	133.9
U2731b	A[r]	134.5	134.3	134.9	A	133.7
U2736b	A[r]	134.4	134.2	135.4	A	133.8
U2741b	A	134	134	134.8	A	134.0
U2746b	--[2]	--	101.4	102.5	B[3]	102.4
U2751b	--	--	101.2	102.2	B[3]	102.2
U2756b	--	--	101.3	102.3	B[3]	102.5
U2761b	--	--	101.5	102.2	B[3]	102.4
U2766b	--	--	101.5	102.4	B[3]	102.4
U2771b	C[Rr]	117.2	117.2	116.9	C	117.4
U2776b	C[r]	117.3	117.2	117.3	C	117.5
U2781b	--	--	116.5	117.5	C	117.36
U2786b	C	117.3	117.2	117.4	C	117.2
U2791b	D	115.6	115.4	117.4	D	115.7
U2796b	C[r]	117.3	117.3	117.4	C	117.7
U2801b	D[rf]	115.6	115.4	116.8	D	113.9
U2806b	D	115.7	115.6	116.7	D	115.6
U2811b	D[rf]	115.6	115.2	117.4	D	115.5
U2816b	D	115.6	115.6	116.9	D	115.6
U2821b	C	117.4	117.1	117.4	E	122.0
U2826b	D[rf]	116.9	116.8	116.8	E	122.0
U2831b	C[Rr]	117.2	117	116.8	E	122.0
U2836b	C[Rr]	117.2	116.9	116.9	E	122.7
U2841b	C[r]	117.4	117	117.1	E	122.7
U2846b	--	--	--	64.78	F	70.63
U2851b	--	--	--	65	F	67.64
U2856b	--	--	--	64.69	F	67.81
U2861b	--	--	--	64.62	F	67.92
U2871b	--	--	--	64.69	F	67.73

[1]Classification based on region I, except for those marked with a superscript, indicating that the discrimination criteria defined in terms of range (R), spectral residual (r), or factor scores (f) were only met when classification was based on regions II.
[2]Sample rejected by the calibration model as not belonging to the population represented by the training set.
[3]Sample reported by the processor to have been incorrectly blended

calibrated. The five samples of type F were all correctly rejected by each of the individual calibration models as well as the combined calibration model. However, the FT-NIR method classified four samples of type E as oil type C and the fifth one as oil type D. In addition, the FT-NIR-predicted IVs for the samples of oil type E are 5 IV units lower than the GC-determined IV reported by the processor, in contrast to the generally good agreement overall between the FT-NIR and GC values shown in Table 5.4 and examined in more detail below. To investigate the reasons for this discrepancy, we determined the IVs of these samples, as well as those of nine samples of oil types A-D, using a Fourier transform mid-IR (FTIR) method previously developed in our laboratory (9). We also analyzed two samples of oil type E and one sample of oil type C by the AOCS iodometric method (14). The results of these various analyses corroborated the FT-NIR predictions, indicating that the GC IVs for the samples identified as belonging to oil type E are incorrect. Accordingly, it may be concluded that the samples provided to us may not have been the same as the ones analyzed by the processor, accounting for the seemingly incorrect classification of these five samples by the FT-NIR method.

For the 20 samples belonging to oil types A-D, the predicted IVs obtained from the individual calibration for the assigned oil type, the combined calibration, and the global calibration were compared to the IVs obtained by GC analysis. The results showed that the individual, combined and global calibrations yielded MD of 0.32, 0.04 and 0.83 IV units, respectively, and SDD of 0.56, 0.51, and 0.87, respectively, relative to the GC data. These results are similar to those shown in Table 5.3 for the initial validation set and again illustrate that slightly better matching of the GC data is achieved using the oil-specific calibrations as opposed to the global calibration.

In our previous work (11), we demonstrated the utility of PLS as a quantitative analysis tool for the prediction of IV as well as *cis* and *trans* content of fats and oils by FT-NIR spectroscopy. In the present work, we have demonstrated that PLS calibrations can also be employed to classify samples among defined oil types. We also have shown that when IV calibrations are specifically developed for defined oil types, the agreement between the NIR predictions for unknowns and the data obtained by the reference method is better

than that obtained from a global calibration based on a broad range of calibration standards. The global calibration was able to predict all the samples provided for this study without flagging them as outliers. For the analytical performance of the global calibration to be comparable to that of the specific calibrations, a validation process is required to determine the bias and correct it. This study has demonstrated that FT-NIR oil analysis, when fully exploiting the combined predictive and discriminant capabilities of PLS, is a powerful and practical analytical quality control tool.

5.5 Acknowledgements

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5.6 References

1. Bewig, K. M., Clarke, A. D., Roberts C. and Nan Unklesbay, Discriminant Analysis of Vegetable Oils by Near-Infrared Reflectance Spectroscopy, *J. Am. Oil Chem. Soc.* 71:195-200 (1994)

2. Sato, T., Application of Principal-Component Analysis on Near-Infrared Spectroscopic Data of Vegetable Oils for Their Classification, *J. Am. Oil Chem. Soc.* 71:293-298 (1994)

3. Wesley, I. J., F. Pacheco, and A.E.J. McGill, Identification of Adulterants in Olive Oils, *J. Am. Oil Chem. Soc.* 73:515-518 (1996)

4. Wesley, I. J., R.J. Barnes, and A. E. J. McGill, Measurement of Adulteration of Olive Oils by Near-Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 72:289-292 (1995)

5. van de Voort, F.R., J. Sedman, G. Emo and A.A. Ismail, Rapid and Direct Iodine Value and Saponification Number Determination of Fats and Oils by Attenuated Total Reflectance/Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 69:1118-1123 (1992)

6. Ismail A.A., F.R. van de Voort, J. Sedman, and G. Emo, Rapid Quantitative Determination of Free Fatty Acids in Fats and Oils by FTIR Spectroscopy, *J. Am. Oil Chem. Soc.* 70:335-341 (1993)

7. van de Voort, F.R., A.A. Ismail, J. Sedman, J. Dubois and T. Nicodemo, The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 71:921-926 (1994)

8. van de Voort, F.R., A.A. Ismail, J. Sedman, and G. Emo, Monitoring the Oxidation of Edible Oils by FTIR Spectroscopy, *J. Am. Oil Chem. Soc.* 71:243-253 (1994)

9. van de Voort, F.R., A.A. Ismail, and J. Sedman, A Rapid Determination of *cis* And *trans* Content of Fats and Oils by FTIR Spectroscopy, *J. Am. Oil Chem. Soc.* 72:873-880 (1995)

10. Dong, J., Kangming Ma, F.R. van de Voort, and A.A. Ismail, Stoichiometric Determination of Hydroperoxides in Oils by Fourier Transform Near-Infrared Spectroscopy, *JAOAC Int.* 80:345-353 (1997)

11. Hui Li, F.R. van de Voort, J. Sedman, and A. A. Ismail, Rapid Determination of *cis* and *trans* Content, Iodine Value and Saponification Number of Edible Oils by Fourier Transform Near-Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 76:491-497 (1999)

12. Youden, W.J., and E.H. Steiner, *Statistical Manual of the AOAC*, Association of Official Analytical Chemists, Arlington, pp18-19 (1975)

13. A Global Iodine Value Calibration for the *Oil and Fat Analyzer*, Application Note, Bomem Inc., Quebec, Canada (1999).

14. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., American Oil Chemists' Society, Champaign, Illinois (1989) Method Cd 1-25

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The ability of the generalized FT-NIR IV calibration to analyze processed edible oils accurately led to considering the development of a generalized *trans* calibration. *Trans* analysis has become increasingly important since legislation was tabled by the US Food and Drug Administration which proposes new *trans* nutritional labeling regulations. The samples provided by Lipton, for which *trans* information was also available, were used to validate the generalized *trans* calibration developed. The discriminate capabilities of PLS developed in Chapter 5 were employed to provide an increased level of confidence in the predictions obtained, a factor crucial to implementing such methodology in an industrial situation.

CHAPTER 6 TRANS DETERMINATION OF EDIBLE OILS BY FOURIER TRANSFORM NEAR-INFRARED (FT-NIR) SPECTROSCOPY

6.1 Abstract

A generalized partial-least-squares (PLS) calibration for determination of the *trans* content of edible fats and oils by Fourier transform near-infrared (FT-NIR) spectroscopy with the use of 8-mm disposable glass vials for sample handling and measurement was developed. The *trans* contents of a broad range of oils were determined using the AOCS single-bounce horizontal attenuated total reflectance (SB-HATR) mid-infrared spectroscopic procedure, these *trans* reference data being used in the development of the generalized FT-NIR calibration. Additional refined and product-specific calibrations were also developed, and all the calibrations were assessed for their predictive capabilities using two sets of validation samples, one comprising a broad range of oil types and the other restricted to oils with specific characteristics. The FT-NIR *trans* predictions obtained using the generalized calibration were in good agreement with the SB-HATR results, the values were accurate and reproducible to within ±1.1 and ±0.5 % *trans*, respectively, compared to a reproducibility of ±0.40 % *trans* obtained for the SB-HATR method. The accuracy of the predictions obtained from the generalized FT-NIR calibration for particular oil types was not significantly improved by supplementing the base training set with samples of these specific types. Calibrating only these oil types did, however, produce a substantial improvement in predictive accuracy, approaching that of the SB-HATR method. These product-specific calibrations produced serious predictive errors when non-representative samples were analyzed. The incorporation of a supplementary discriminate analysis routine was found to be a powerful safeguard in flagging non-representative samples as outliers and could also be used to select the calibration most appropriate for the characteristics of the sample being analyzed. Overall, it was concluded that FT-NIR spectroscopy provides a viable alternative to the SB-HATR/mid-FTIR method for *trans* determination, making use of more industrially robust instrumentation and equipped with a simpler sample handling system.

KEY WORDS: Edible oil analysis, Fourier transform near-infrared spectroscopy, FT-NIR, partial least squares, PLS, *trans* content

6.2 Introduction

Ttrans fatty acids (TFA) in edible fats and oils have been implicated in the etiology of arteriosclerosis and heart disease (1,2) and the U.S. Food and Drug Administration (FDA) has recently published a proposed amendment of their regulations on nutritional labeling (3). This will require that the amount of *trans* fatty acids in foods, as well as dietary supplements to be included in the amount and percent daily value declared for saturated fatty acids. TFAs are commonly produced from unsaturated fatty acids during hydrogenation in a concurrent side reaction, in which some of the *cis* double bonds are incompletely hydrogenated and converted to the *trans* configuration instead. The degree of hydrogenation, as well as the levels of TFA formation, are key determinants of the quality and functionality of fats and oils, and monitoring of TFA formation is a useful way of tracking and controlling the hydrogenation process.

Mid-infrared spectroscopy has been the predominant means of determining the *trans* content of fats and oils since 1940's (4). The advent of Fourier transform infrared (FTIR) spectroscopy has led to substantive improvements in compensating for interfering triglyceride absorptions, eliminating the need to saponify and methylate the oils to produce fatty acid methyl esters (5). A related infrared spectroscopic technique, near-infrared (NIR) spectroscopy, is widely used in the food industry for quality control applications, being a rapid, reliable and nondestructive means of analyzing various materials, without reagents or solvents. NIR instruments are more rugged and less energy-limited than mid-IR spectrometers, and offer more flexible sample handling options, making it possible to analyze samples in low-cost and convenient glass vials. These advantages are also inherent to FT-NIR spectrometers, with the added benefit of highly repeatable measurements owing to the use of a Helium-Neon reference laser to ensure wavelength accuracy. Wavelength accuracy is the key to obtaining robust calibrations and facilitates the transfer of calibrations from one instrument to another, generally a problematic process with traditional grating or filter-based instruments.

The McGill IR Group has worked extensively on the development of rapid FTIR methods for the analysis of edible oils, with a focus on mid-FTIR spectroscopy. One of the first *trans* analysis methods developed was part of a mid-FTIR edible oil analysis package that simultaneously allowed the determination of *cis* and *trans* content as well as iodine value (IV) and saponification number (SN) using PLS calibrations based on pure triglyceride standards (6). Subsequently, it was shown that a FT-NIR spectrometer equipped with a 5-mm quartz transmission cell could be used to accurately determine *cis*/*trans*/IV/SN when calibrated using mid-FTIR spectroscopic data (7). Bomem, an FTIR instrument manufacturer with an interest in developing practical oil analysis instrumentation, developed a "global" FT-NIR IV calibration for 8-mm glass vials based on a broad variety of oil samples, the reference values for this calibration being provided by GC (8). Subsequently, more accurate oil specific IV calibrations were developed and employed using discrimination criteria based on various types of information provided by these calibrations (9). This work led to the conclusion that a generalized, instrument-transferable, FT-NIR IV calibration would provide sound IV analytical data and that the calibration could be further refined with processor-specific oils if additional accuracy was needed. A NIR IV method based on the use of a global calibration is being considered by the AOCS for official method status.

This paper expands on the concepts developed as a result of the IV work. The objective of this research was to develop a generalized *trans* calibration for a FT-NIR spectrometer, equipped with a heated sample-handling accessory that uses 8-mm disposable vials. The overall goal is to provide processors with a rapid and simple means of determining *trans* content with instrumentation more suited to the industrial quality control environment.

6.3 Materials and Methods

6.3.1 Oil Samples, Calibration and Validation Standards

Oil samples representing a broad range of oil types and degrees of hydrogenation were obtained from California Oils Co. (Richmond, CA), Bunge Foods (Bradley, IL) and Lipton (Baltimore, MD). The reference *trans* values (expressed as % trielaidin) for all the

California/Bunge group (CBG) oils were obtained using the AOCS single-bounce horizontal attenuated total reflection mid-IR method (5). For the calibration of the reference method, triolein and trielaidin were obtained from Nu-Chek Prep, Inc. (Elysian, MN) and an ultra-degummed bleached expeller soybean reference oil was obtained from Owensboro Grain Co. (Owensboro, KY). In the case of the Lipton group (LG) samples, *trans* data obtained by the new AOCS GC method (5) were provided by the processor.

The training set for the generalized *trans* calibration consisted of 44 CBG oil samples, which included, among others, undefined commercial shortenings and palm, corn, almond, soybean, canola, and walnut oils, both hydrogenated and nonhydrogenated. Figure 6.1 provides a frequency histogram of the *trans* content distribution in the training set, which ranged from ~0 to 50% *trans*. The LG samples consisted of four sets of 40 preanalyzed, but undeclared oil types, only identified by being grouped as A-D (Table 6.1). A second *trans* calibration set was prepared by adding 20 LG samples, five of each type, to the 44-sample base training set used for the general calibration. Additional calibrations were developed for each specific LG oil type (A-D) by using 20 samples of each group to develop individual group calibration training sets as well as a combined LG calibration training set consisting of 5 samples from each oil type. The makeup of the various calibration training sets is summarized in Table 6.2. Two sets of validation samples were used to evaluate all the calibrations. None of these validation samples were used in any of the calibration training sets. The first validation set (Val-1) consisted of 21 samples selected from the CBG oil group while the second set (Val-2) consisted of 20 LG samples obtained from oil groups A-D (five from each).

6.3.2 Instrumentation and Sample Handling

A Bomem (Bomem Inc., Quebec, Canada) MB155S FTIR spectrometer, configured for near-IR operation and equipped with a deuterated triglycine sulfate (DTGS) detector, was used for this study. The spectrometer was controlled by an IBM-compatible 486 DX 66-MHz PC running under Windows-based Bomem-Grams/386 software (Galactic

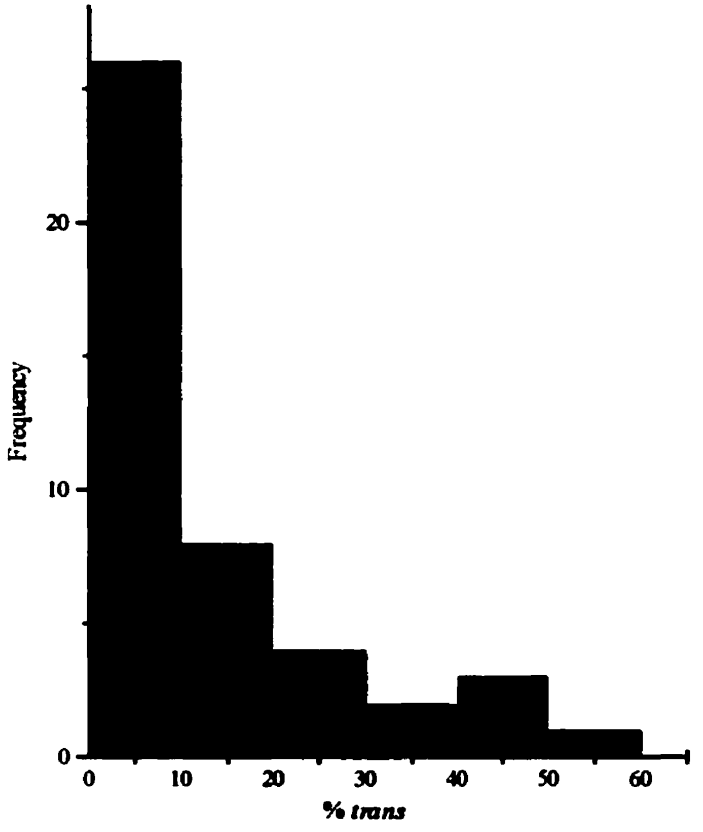

Figure 6.1. A frequency distribution plot illustrating the *trans* values of the samples in the training set used to derive the generalized calibration (Cal-1).

Table 6.1. Mean *trans* values, their standard deviations and ranges for oil samples within Lipton (LG) oil groups A-D

Oil Type	% *trans*		
	Mean	SD	Range
A	0.567	0.099	0.33–0.71
B	26.921	1.207	25.20–28.81
C	8.893	0.714	7.80–10.17
D	8.898	0.656	7.95–10.12

Table 6.2. Summary of the sample makeup of the training sets used for the various *trans* calibrations

Calibration	CGB[1] Oils	LG[2] Oils
Cal-1	44 samples	0 samples
Cal-2	44 samples	20 samples (5 each of oil types A, B, C, and D)
Cal-3	0 samples	20 samples (5 each of oil types A, B, C, and D)
Cal-4a	0 samples	20 samples of oil type A
Cal-4b	0 samples	20 samples of oil type B
Cal-4c	0 samples	20 samples of oil type C
Cal-4d	0 samples	20 samples of oil type D

[1]California/Bunge Group
[2]Lipton Group

Industries Co., Salem, NH) and AIRS, a specific quality assurance software package (DHC Analysis, Cleveland, OH). A Nicolet (Madison, WI) Magna FTIR spectrometer operating under Nicolet OMNIC E.S.P. software and equipped with a heated ZnSe SB-HATR accessory (Graseby-Specac, Smyrna, GA) was employed for the determination of the reference *trans* values by AOCS Official Method Cd 14d-97 (5).

For acquisition of FT-NIR spectra, samples were transferred to 8-mm o.d. transparent glass vials (Kimble Glass Inc., Vineland, NJ) having a nominal volume of ~1 mL. The vials were placed in a temperature-controlled, multivial sample holding block (Bomem Inc.) maintained at 68 ± 1°C. All sample and background spectra were recorded by co-adding 128 scans at a resolution of 16 cm^{-1} over the spectral range of 10,000-4000 cm^{-1}. Open-beam background spectra were collected every 30 min with the vial holder in the IR beam, and each sample spectrum was ratioed against the most recent background spectrum collected. To compensate for inherent variations in vial pathlength, the ratioed spectra were normalized to unit area over the region of 9100-7560 cm^{-1} by using a normalization routine provided with the AIRS software.

6.3.3 Near-IR Calibration and Validation

The spectra of the *trans* calibration standards along with their respective reference *trans* values were input into the Galactic PLSPlus chemometrics program (Galactic Industries Co.) to develop PLS calibrations. Correlation and variance spectra were generated to identify spectral regions that contain information related to variations in *trans* content, and these regions were then explored for calibration development and refinement. Each calibration was assessed by using the leave-one-out cross-validation procedure, and optimized in terms of the appropriate number of factors by minimizing the predicted residual error sum of squares (PRESS). The performance of the calibrations, as well as their respective cross-validations, was assessed using linear regression, with accuracy (a) and reproducibility (b) calculated as mean differences (MD) and standard deviations of the differences (SDD) for r or a according to the method of Youden (10).

6.4 Results and Discussion

6.4.1 SB-HATR Results

The calibration of any secondary method, such as an FT-NIR *trans* method, is strongly dependent on the accuracy and reproducibility of the reference method upon which it is based. The new AOCS SB-HATR mid-FTIR *trans* method (5) was used to obtain the reference *trans* values for the CBG oil samples. The SB-HATR method itself requires calibration based on the use of two-component gravimetrically prepared mixtures of trielaidin and triolein. This method entails ratioing of the spectra of these standards, as well as those of the samples subsequently analyzed, against the spectrum of a zero-*trans* reference oil, with quantitation being based on measurement of the area under the isolated *trans* absorption band between 990 and 945 cm^{-1} in the ratioed spectra. The calibration equation obtained by linear regression of the mean area from duplicate spectra of the calibration standards vs. the gravimetric *trans* content was:

$$\text{Area} = 0.37008 + 0.38121(\%\ trans) \quad r^2 = 0.997 \qquad SD = 0.58 \qquad [6.1]$$

Subsequently, the 44 oil samples constituting the training set for the FT-NIR calibration were analyzed in duplicate by the SB-HATR method and their *trans* contents were predicted using equation [6.1]. The duplicate predictions are plotted against each other in Figure 6.2; the equation for the line is:

$$\%\ trans_1 = 0.0181 + (1.0086 * \%\ trans_2) \quad r^2 = 0.999 \qquad SD = 0.40 \qquad [6.2]$$

The SD associated with equations [6.1] and [6.2] indicate that the reference method can be reproducible to within ±0.40% *trans* and accurate to ±0.60% *trans*. For comparative purposes, selected LG samples, analyzed by GC, were also analyzed by the SB-HATR method, and the GC *trans* data were regressed against the FTIR-predicted *trans* contents to obtain an estimate of their relative degree of concurrence, which is summarized by equation [6.3].

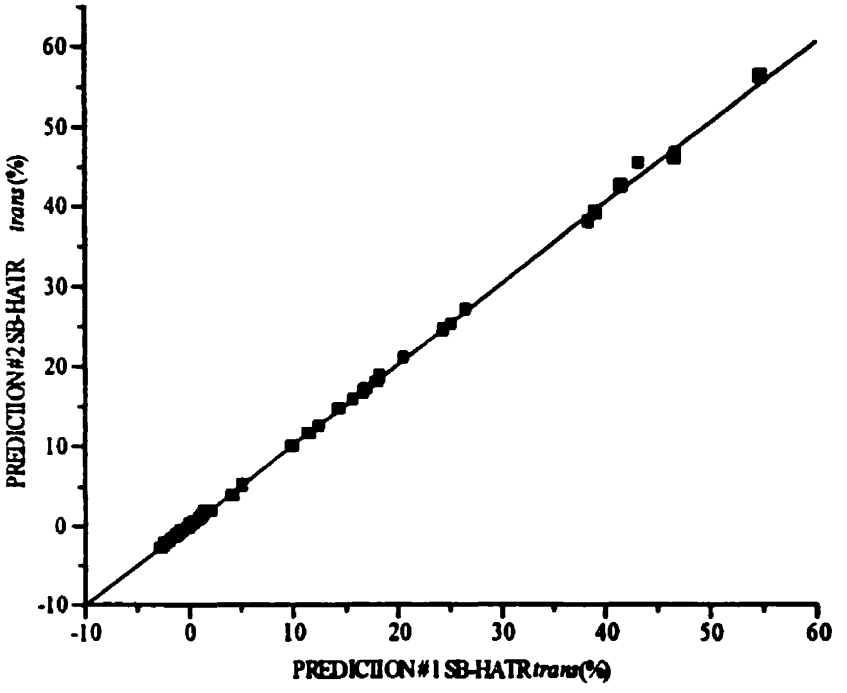

Figure 6.2. **Duplicate predictions of CGB calibration standards by SB-HATR method**

$$\text{GC \% } trans = 0.24 + 0.975 \text{ SB-HATR \% } trans \qquad r^2=0.998 \quad SD = 0.92 \qquad [6.3]$$

Equation [6.3] indicates that the LG GC data, obtained using the new AOCS GC method (5), are a remarkably good match to the SB-HATR data, corroborating the observations of Adam *et al.* (11) that general concurrence between GC and IR *trans* data is finally being achieved.

6.4.2 Spectroscopy and Calibration Development/Assessment

Figure 6.3 illustrates the spectral characteristics of a low-*trans* and a high-*trans* edible oil across the NIR and mid-IR regions of the spectrum. Owing to the low intensity of NIR absorption in comparison with mid-IR absorption, the absorbance in the NIR region (10,000-4000 cm^{-1}) has been magnified 10-fold to allow detail to be seen. The bands in the NIR region are broad and nebulous in comparison with the detailed structure observed in the mid-IR region. What is particularly noteworthy about these spectra, in relation to the quantitation of *trans* content, is the lack of any discernable difference between the spectra of the low- and high-*trans* oils in the NIR region, whereas the mid-IR portion of the spectrum shows a clear-cut, strong *trans* signal at 967 cm^{-1}. Although the NIR region contains much of the same information as the mid-IR region, extensive band overlap makes it more difficult to extract this information since the NIR bands correspond to overtones and combinations of the fundamental vibrations that give rise to mid-IR absorption bands. As a result, in most cases the only means of carrying out quantitative work in the NIR region is to make use of more sophisticated chemometric techniques, such as (PLS), which provide a means of developing a calibration by mathematically correlating spectral changes to changes in the measure of interest. In the present work, we have employed PLS to derive a generalized FT-NIR calibration for the prediction of *trans* content.

The FT-NIR spectra of the samples in the Cal-1 training set (Table 6.2) were input, along with their SB-HATR reference *trans* values into a chemometrics program to develop a generalized PLS calibration. Correlation and pure-component spectra as well as PRESS and cross-validation results indicated that the regions selected in our initial study

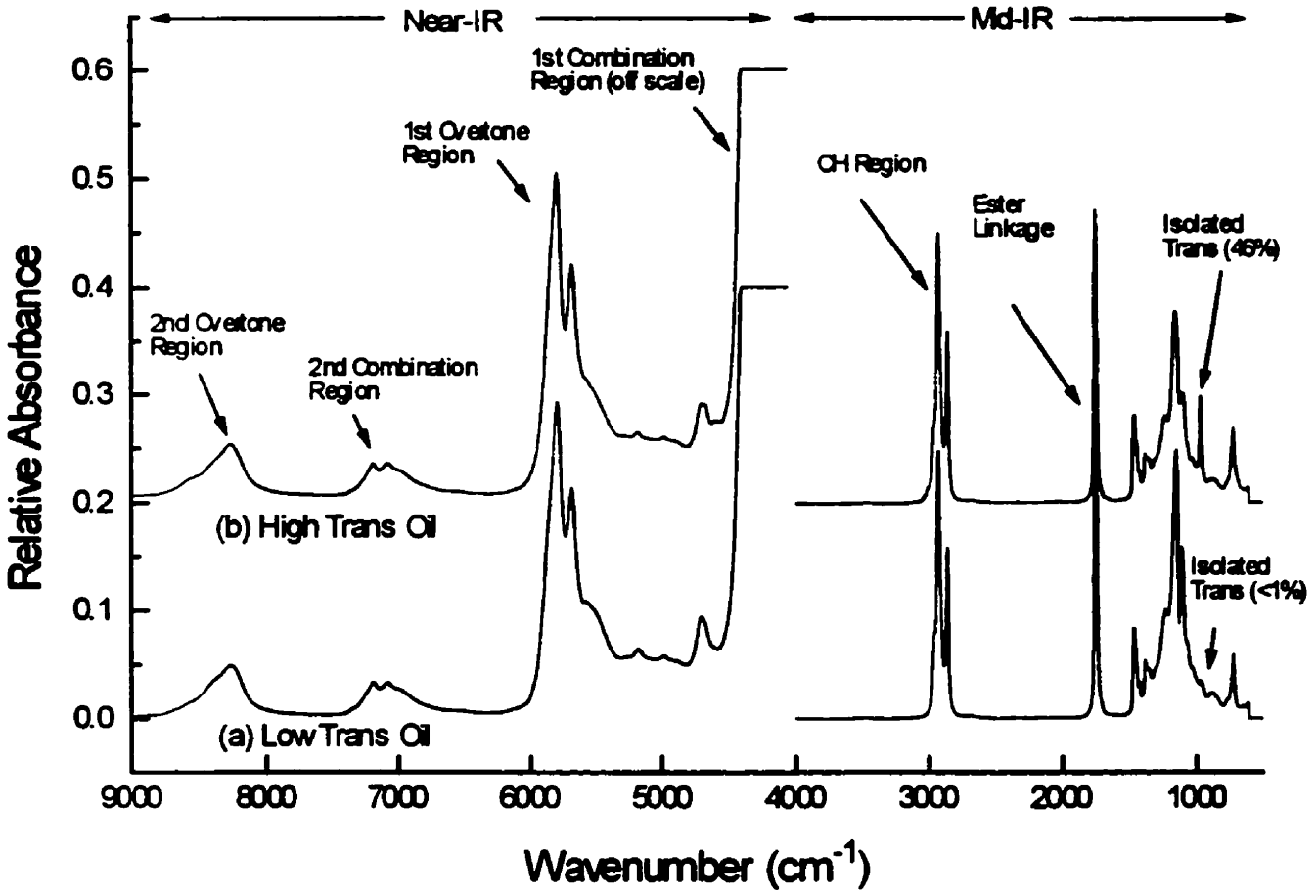

Figure 6.3. Collated near/mid-IR spectra of (a) a low-*trans* oil and (b) a high-*trans* oil, illustrating the key spectral features associated with the two IR spectral regions. The fundamental *trans* absorption at 967 cm^{-1} is readily discernable in the mid-IR region. The absorbance values in the region from 10,000 to 4000 cm^{-1} have been magnified by a factor of 10 to provide greater detail.

(7) were optimal for the determination of *trans* content. The optimal spectral regions were found to be 8975-7189 cm^{-1} with a single-point baseline at 7575 cm^{-1}, 5238-5056 cm^{-1} with a single-point baseline at 5247 cm^{-1}, and 4779-4564 cm^{-1} with a single-point baseline at 4800 cm^{-1}. The Cal-1 PLS model used five factors and Figure 6.4 illustrates the cross-validation plot of FT-NIR predicted *trans* vs. SB-HATR *trans* content, expressed as % trielaidin with its linear regression equation being:

$$\text{FT-NIR}trans = 0.17 + 0.99*\text{SB-HATR}trans \quad r^2 = 0.995 \quad SD = 1.51 \qquad [6.4]$$

The MD_a and SDD_a for the predictions from the Cal-1 calibration relative to the ATR *trans* values were -0.05 and 1.51, respectively, quite similar to the results obtained in our first FT-NIR *trans* study (7). In that study, as in the present work, the reference values were obtained by mid-FTIR spectroscopy; however, a PLS-based method using a 25-μm transmission flow cell was employed instead of the SB-HATR method. The comparable accuracy obtained in the two studies indicates that the performance of the two mid-FTIR *trans* methods is essentially equivalent, such that either can serve as a reference method in the development of an FT-NIR calibration. It also indicates that the use of glass vials for sample handling, with the use of a spectral normalization procedure to compensate for their inherent pathlength variability (9), does not appear to detract from the general performance of the FT-NIR predictive capability relative to the use of a constant pathlength transmission cell.

Although the FT-NIR predictions obtained clearly track the reference values (Figure 6.4), the overall cross-validation SD is 1.5% *trans*, about three times that of the *r* of the SB-HATR reference method. Cal-1 was subsequently validated using two sets of validation oils, Val-1 (CBG samples) and Val-2 (LG samples). Table 6.3 illustrates the MD and SDD obtained for both accuracy and reproducibility of the FT-NIR predictions for these two validation sets relative to their respective reference methods. Overall, the SDD_r for duplicate FT-NIR *trans* predictions is very similar to that of the reference SB-HATR method. The SDD_a reflects a somewhat better accuracy than indicated by the cross-validation results (±1 vs. ±1.5), most likely because the calibration training set

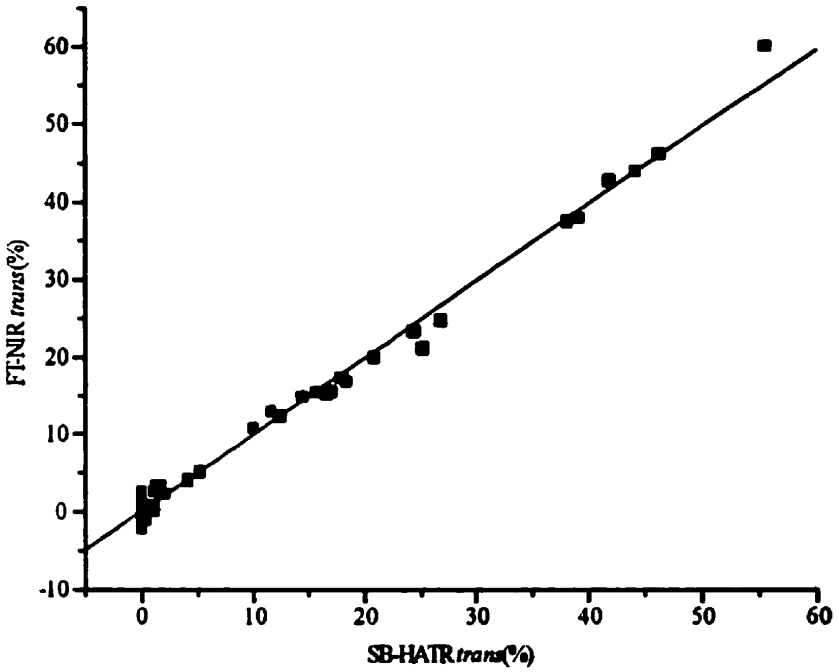

Figure 6.4. A cross-validation plot of Foutrier transform near-infrared (FT-NIR) *trans* predictions obtained using the Cal-1 vs. the single bounce horizontal attenuated total reflectance (SB-HATR) *trans* reference values.

Table 6.3. Mean differences (MD) and standard deviations of the differences (SDD) for reproducibility (r) and accuracy (a) obtained for samples analyzed with the general (Cal-1) and the refined (Cal-2) *trans* calibrations as assessed using the two validation sets (Val-1 and Val-2)

Validation Set	Reference Method	Cal-1 Statistics	Cal-2 Statistics
Val-1 N = 21 CBG[1] Oils	AOCS SB-HATR	MD_r = -0.08 SDD_r = 0.55 MD_a = -0.06 SDD_a = 1.13	MD_r = -0.15 SDD_r = 0.42 MD_a = 0.02 SDD_a =1.12
Val-2 N = 20 LG[2] Oils	AOCS GC	MD_r = -0.04 SDD_r = 0.38 MD_a = -0.15 SDD_a = 1.11	MD_r = -0.05 SDD_r = 0.38 MD_a = -0.27 SDD_a = 1.01

[1]California/Bunge Group
[2]Lipton Group

spanned a wider range of *trans* values than the validation samples. The similarity of the SDD_a values for the two validation sets provides further evidence of the general consistency between the AOCS SB-HATR and AOCS-GC reference methods. The SDD_a values are about twice the SD for the SB-HATR method in equation [6.1]. This somewhat poorer accuracy is not surprising given the need to extract the *trans* spectral information from weak overtone and harmonic bands rather than the sharp, fundamental band present in the mid-IR region.

In this context, we also evaluated whether Cal-1 could be further refined to better predict LG type samples by the inclusion of 20 additional representative samples in the training set (Cal-2). The MD and SDD statistics for this calibration are presented together with those for Cal-1 in Table 6.3. The accuracy of the predictions for the Val-2 set did not improve appreciably with the inclusion of LG samples in the training set, implying that the training set for Cal-1 modeled the LG samples adequately. Subsequently, a calibration was developed specifically for the LG oils. Five samples of each oil type, for a total of 20 samples, were used to develop the calibration (Cal-3). The reference values for this calibration were provided by GC analyses, and the optimized Cal-1 spectral regions were employed. Cal-3 was used to predict Val-1 and Val-2 and the prediction statistics, in terms of MD and SDD for reproducibility and accuracy, are given in Table 6.4. As expected, since Cal-3 is specific to LG samples, it is useless for predicting Val-1 oils. However, Cal-3 does a very good job of predicting the *trans* values for the Val-2 (LG) samples. The SDD_a is about ±0.50% *trans*, significantly better than ±1.1% *trans* for Cal-1.

6.4.3 Discriminate Analysis

When implementing a PLS calibration, the PLS-predicted values for samples whose spectra differ significantly from those of the training set cannot be considered reliable. This is well illustrated by the results presented above for Cal-3. Because the training set for this calibration was restricted to four specific oil types (LG oil types A-D), the *trans* predictions obtained for Val-1 oils were off by 20% or more. Similarly, if a sample

Table 6.4. MD and SDD for reproducibility and accuracy obtained for samples analyzed using the general Lipton group calibration (Cal-3) using the two validation sets (Val-1 and Val-2)

Validation Set	Reference Method	Cal-3 Statistics	
Val-1 N = 21 CBG[1]	AOCS SB-ATR	MD_r = -0.76 MD_a = -14.11	SDD_r = 0.91 SDD_a = 17.30
Val-2 N = 20 LG[2]	AOCS GC	MD_r = 0.18 MD_a = 0.48	SDD_r = 0.61 SDD_a = 0.62

[1]California/Bunge Group
[2]Lipton Group

were atypical in any way (e.g., containing excess free fatty acids or heavily oxidized relative to the samples in the training set), it may not be accurately predicted. Thus, practical implementation of a PLS calibration should incorporate some means for detecting "outliers", i.e., samples that are not well represented spectroscopically by the samples used to derive the calibration.

The AIRS software includes discriminate analysis capability, which can be used to determine whether spectra of samples are consistent with the characteristics of the calibration training set. As detailed in a previous paper (9), the use of discriminate analysis for outlier detection can be based on a variety of criteria, including the PLS-predicted value of the parameter of interest, the spectral residual, or the factor scores. These three types of discrimination criteria were devised for Cal-1. Based on these criteria, none of the samples in Val-1 and Val-2 were identified as outliers. This provides further evidence that the Val-2samples were adequately modeled by the general Cal-1 training set. Refining this calibration by the addition of LG samples to the training set (Cal-2) did not provide a significant improvement.

In the case of Cal-3, all the samples in Val-1 were flagged as outliers when tested against the discrimination criteria established for this calibration, as would be expected on the basis of the makeup of the training set (Table 6.2). All the samples in Val-2 passed the Cal-3 discrimination criteria. Subsequently, four calibrations, Cal-4 (a-d), were developed specifically for each LG oil type and discrimination criteria were set to determine whether the AIRS discriminate analysis routine could distinguish among the four oil types. Since the *trans* ranges of oil types C and D overlap appreciably (see Table 6.1), difficulty in distinguishing between oils of these two types might be anticipated. However, the AIRS discriminate analysis routine was readily able to distinguish among samples of all four oil types. This capability could be very useful as it not only provides a means of ensuring that the calibration in place is appropriate for the sample being analyzed, but also can be used to automate the selection of the most appropriate calibration from among a set of available calibrations. The AIRS software tests the sample spectrum using the discrimination criteria for each calibration and uses this information to select the most

appropriate calibration to predict the *trans* content of the sample. Thus, samples of different oil types can be analyzed in random order without any need for operator intervention to select an appropriate calibration.

Based on our experience to date (9), we are finding that discriminate analysis is a very powerful and important tool, which, in our opinion, should be an integral part of any IR spectroscopic analytical method to be implemented in an industrial situation. The application of discriminate analysis routines helps eliminate errors such as mixing up a sample or analyzing one that is spoiled or contaminated, thereby providing an extra degree of confidence in the analytical results.

6.5 Conclusion

This study demonstrates that despite th e lack of a discernable *trans* absorption band in the NIR portion of the spectrum, the *trans* content of edible fats and oils can be predicted from their FT-NIR spectra through the application of PLS. By employing a training set comprising of a wide variety of oil types, a generalized calibration was developed that allowed the *trans* content of unknown samples, analyzed in disposable glass vials, to be predicted with an accuracy of ~±1.1% *trans*. Supplementing the calibration training set with a subset of a specific oil type or group does not appear to improve the accuracy of the basic calibration. On the other hand, it is possible to obtain more accurate predictions by calibrating on a more limited training set of oils having specific characteristics (e.g., canola oils undergoing a standardized hydrogenation process). These more restricted calibrations are strictly limited to oils having the characteristics reflected by the training set, and discriminate analysis is an important supplementary tool for these types of calibrations because it provides the capability to flag samples that do meet this requirement. The generalized FT-NIR *trans* calibration does not match the performance characteristics of the mid-FTIR SB-ATR method, but when specific calibrations are developed for well-defined oil types, the accuracy approaches that of the mid-FTIR method. NIR spectroscopy is clearly a competitive, alternative approach to mid-IR spectroscopy for the rapid determination of the *trans* content of fats and oils. It has the

advantage of using more robust instrumentation and a simpler sample handling system better suited to the industrial QC environment. Supplemented with the AIRS discriminate analysis package, the FT-NIR system evaluated provides a practical means for the edible oil sector to comply with the new *trans* labeling requirements recently proposed by the FDA.

6.6 Acknowledgments

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6.7 References

1. Haumann, B.F., Dietary Fats Still Spark Controversy, *INFORM* 5:358 (1994).

2. Aro, A., Epidemiological Studies of *trans* Fatty Acids and Coronary Heart Disease, in *Trans Fatty Acids in Human Nutrition*, edited by J. L. Sebedio and W. W. Christie, The Oily Press, Dundee, Scotland, pp235-260 (1998)

3. *Federal Register*, Vol. 64, No. 221 (Nov., 17, 1991)

4. *Official and Tentative Methods of the American Oil Chemists' Society*, edited by V.C. Mehlenbacher, American Oil Chemists' Society, Chicago (1946) Tentative Method Cd 14-61

5. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by D. Firestone, 5th edition, AOCS Press, Champaign (1998) Method Ce 1g-96, Cd 14d-97, and Ce 1f-97

6. van de Voort, F. R., A. A. Ismail and J. Sedman, A Rapid Automated Method for the Determination of *cis* and *trans* Content of Fats and Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 72:873-880 (1995)

7. Li, H., F. R. van de Voort, J. Sedman, and A. A. Ismail, Rapid Determination of *cis* and *trans* Content, Iodine Value, and Saponification Number of Edible Oils by

Fourier Transform Near-Infrared Spectroscopy, *J. Am. Oil Chem. Soc.* 76:491-497 (1999)

8. Anonymous, A Global Iodine Value Calibration for the Oil and Fat Analyzer, Application Note ABB Bomem Inc. Quebec, Canada. (1999)

9. Li, Hui, F. R. van de Voort, A. A. Ismail, J. Sedman, R. Cox, C. Simard, and H. Buijs, Discrimination of Edible Oil Products and Quantitative Determination of Their Iodine Value by FT-NIR Spectroscopy, *J. Am. Oil Chem. Soc.* 77:29-36 (2000)

10. Youden, W. J. and E.H. Steiner, *Statistical Manual of the AOAC*, Association of Official Analytical Chemists, Arlington, VA (1975)

11. Adam, M., M. M. Mossoba, T. Dawson, M. Chew, and S. Wasserman, Comparison of Attenuated Total Reflection Infrared Spectroscopy to Capillary Gas Chromatography for *trans* Fatty Acid Determination, *J. Am. Oil Chem. Soc.* 76:375-378 (1999)

CHAPTER 7 GENERAL CONCLUSION

The research described in this thesis was undertaken to develop FT-NIR spectroscopy as a quantitative method applicable to the analysis of fats and oils for process quality control purposes. The foundations for edible oil analysis by mid-FTIR spectroscopy have been developed by the McGill IR Group over the last 10 years, and this work resulted in a variety of viable mid-IR methods. In particular, an FTIR Edible Oil Analysis Package, which allows one to simultaneously determine *cis/trans* content, IV and SN of neat fats and oils, was extensively developed, automated and validated. This analytical package was chosen as a vehicle by which to test the hypothesis that a quantitative method based on the mid-IR region of the spectrum should be transferable to the NIR region. Thus, the first aspect addressed was whether the *cis/trans* content, IV and SN analysis could be performed in the NIR region. As in the mid-IR method, a heated transmission cell was employed, leaving the key variable simply the spectral region from which the calibrations were derived. As described in Chapter 3, FT-NIR PLS calibrations were successfully developed to allow the determination of *cis/trans*, IV and SN values for edible fats and oils with a heated flow cell accessory. The FT-NIR analysis could be carried out at-line, with a total analysis time of about 2 minutes per sample. This work also demonstrated the inherent limitation associated with the use of process samples as standards for the development of PLS calibrations when parameters are highly correlated. Although correlated parameters do not prevent one from calibrating on such samples, it is crucial that the standards used for calibration are representative of the samples to be analyzed and that the limited scope of process calibrations be recognized.

Having established that FT-NIR spectroscopy was suitable for quantitative analysis of fats and oils, the focus shifted to evaluating disposable glass vials as an alternative to cuvettes or flow cells as a sample handling system, using a well-characterized mid-IR PV method as a reference method. It was shown that PVs ranging from 1 to 10 could be accurately measured with a reproducibility of ~±1.0 PV, the key to accurate quantitation being the use of a spectral normalization routine to compensate for the pathlength differences between vials. In contrast to the mid-IR PV method, for which the calibration is oil independent, the NIR calibration is oil dependent and hence not universally

applicable. The scope of the calibration can, however, be maximized by using a broader selection of oil types in formulating the calibration. The pivotal result of this study was that the use of disposable vials should not significantly limit the accuracy of FT-NIR analyses, and this provided the impetus to develop more generalized FT-NIR analyses.

Chapters 5 and 6 extended the applications of the vial sample handling system to FT-NIR methods developed specifically for independent IV and *trans* determinations, respectively. In Chapter 5, PLS calibrations were developed for four oil types, and discrimination criteria that allowed the four oil types to be unambiguously differentiated from each other were established based on the predicted values of IV, the spectral residuals and the PLS factor scores output from the PLS calibration models. Using these criteria, the validation samples provided by an oil processor, including a set of blind validation samples, were all correctly classified, and the FT-NIR IV predictions were in excellent agreement with GC data provided by the processor. Thus, discriminate analysis was demonstrated to be a powerful tool for differentiating between oil types and was shown to work well in selecting appropriate calibrations and avoiding sampling errors. Included as part of this study was the evaluation of a "global" IV calibration based on some 1200 preanalyzed fats and oils, developed by Bomem. This calibration was intended to provide some of the generalized characteristics of the "universal" mid-IR calibration based on pure triglycerides. The FT-NIR global calibration worked well, providing good approximations of IV for a wide variety of oils, although it did not perform as well as the oil-specific calibrations. The positive results obtained for the global IV calibration led to the work described in Chapter 6, which was undertaken to investigate whether a generalized calibration for the determination of isolated *trans* isomers. A *trans* calibration was developed based on a broad range of commercial oils and was shown to perform well with both processor samples and samples that were part of an AOCS collaborative study of the SB-HATR mid-FTIR method. The discriminant features available through PLS were incorporated into the FT-NIR method to select calibrations and detect outliers. The results from the generalized FT-NIR PLS calibration as well as from refined and product-specific calibrations were compared to those obtained using the AOCS SB-HATR/FTIR method. It was found that the FT-NIR *trans* predictions obtained using the generalized

calibration were in good agreement with the SB-HATR/FTIR results, with an accuracy and reproducibility of ±1.1 and ±0.5% *trans*, respectively. It was also found that for any particular oil type, the accuracy of prediction would be improved with oil-specific calibrations, with the supplementary discriminate analysis routines being a powerful safeguard in flagging non-representative samples and selecting the calibration most appropriate to the characteristics of the sample being analyzed.

In combination, the four chapters (publications) comprising this work serve to demonstrate that FT-NIR spectroscopy can serve as a multicomponent, quantitative analytical tool for the fats and oils industry. The FT-NIR method developed for the determination of *cis* and *trans* content, IV and SN in fats and oils, much like the original mid-FTIR method, would be of interest to most processors carrying out refining, blending, hydrogenation or interesterification operations. The downside of this multicomponent analytical method is that it was dependent on mid-IR data for its calibration. The subsequent independent IV and *trans* calibrations developed (Chapters 5 and 6) for specific oil types as well as more generalized calibrations verified that an FT-NIR spectrometer equipped with the vial sample handling accessory is capable of producing quantitative results. The FT-NIR methodology developed in this work was designed to meet the demands made by the fats and oils industry for instrumental methods that overcome the time, hazards and reagent disposal issues associated with traditional wet chemical methods. The development of mid-FTIR methods was the first response to these demands. However, mid-FTIR spectrometers, although superior in performance and sample handling capabilities to traditional dispersive IR spectrometers, are, by and large, not well suited to the rough and tumble world of at- or on-line process control. NIR instruments, on the other hand, have been designed for harsher environments and have proven to be rugged and versatile process control instruments. Industrial application was the main impetus for this research, the overall objective being to draw on concepts, methods, and tools employed in the development of mid-IR edible oil analysis methodology and transfer them to FT-NIR instruments. Although FT-NIR instruments are still relatively new and have never been used for quantitative edible oil analysis, market studies indicated that the instrumentation and in particular the vial sample handling

system would appeal to potential users if key analyses such as IV and *trans* could be developed. The work carried out has enabled the transition from mid-FTIR to FT-NIR spectroscopy, first by developing a FT-NIR *cis/trans*, IV and SN method that parallels the mid-IR method previously developed by the McGill IR Group, then by evaluating the vial sample handling accessory using a well-defined stoichiometric PV method, and finally by producing independent FT-NIR IV and *trans* methods. These methods in turn were further strengthened by implementing the powerful discriminant capabilities of PLS, which should ultimately become a standard feature of any PLS-based method. Discriminate analysis brings with it the ability to select the most appropriate calibration and can provide warnings when samples have spectral characteristics that are not modeled by the calibration.

Based on the work carried out, it is the author's belief that FT-NIR spectroscopy will be a competitive alternative to mid-FTIR spectroscopy because the instrumentation is more robust and the vial sample handling system is cheap, works well and is convenient to use. Ultimately, for an instrumental method to be acceptable for routine quality control work, simplicity of operation is a must since it is important that non-technical individuals be able to carry out the analysis. One of the more tangible results of the work described in this thesis is that ABB Bomem Inc. (Quebec, Canada) is successfully marketing FT-NIR instruments for edible oil analysis, and the Bomem IV analysis is being marketed (Figure 7.1) as child's play (3). Ultimately, behind the scenes this is not the case; but once programmed with the method and the calibration and interfaced for QC, the Bomem "Oil Analyzer" is straightforward to operate.

As a consequence of the work carried out, a structured FT-NIR IV method has also been devised based on the global IV calibration evaluated in Chapter 5. Although not included as a formal part of the thesis, this FT-NIR IV method is presently being considered for official method status by the AOCS based on the results of an extensive international collaborative study involving 10 laboratories in 8 countries (1), including the McGill IR

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Figure 7.1. Bomem's advertisement of FT-NIR IV determination package in *INFORM*.

Group. Should this new method be accepted, it will bring additional credibility to the general concept that FTIR spectroscopy may be the next generation of oil analysis methodology (2). Having demonstrated that quantitative analysis of fats and oils is possible by FT-NIR spectroscopy, the door is now also open to other analyses as well as on-line analysis facilitated by the availability of low-cost NIR-transmitting optical fibers. With AOCS recognition pending for the FT-NIR IV method and active marketing of this new methodology underway, the author has had the fortune of seeing a concept through to practice, making the effort expended very satisfying.

References

1. Bomem, I Just Got My First Iodine Value!, *INFORM* 10:444 (1999)

2. Cox, R., J. Labrassier, E. Michiels, and H. Buijs, Hui Li, F. R. van de Voort, , A. A. Ismail, and J. Sedman, Determination of Iodine Value by Fourier Transform Near-Infrared Spectroscopy – A Collaborative Study, *J. Am. Oil Chem. Soc.* (submitted)

3. van de Voort, F. R. and J. Sedman, FTIR Spectroscopy –The Next Generation of Oil Analysis Methodologies? *INFORM* (in press)