NOVEL APPROACHES TO AUTOMATED QUALITY CONTROL ANALYSES OF EDIBLE OILS BY FOURIER TRANSFORM INFRARED SPECTROSCOPY: DETERMINATION OF FREE FATTY ACID AND MOISTURE CONTENT

Ahmed Ali Al-Alawi

September 2005

Department of Food Science and Agricultural Chemistry

McGill University, Montreal

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

Your file Votre référence
ISBN: 978-0-494-25088-4
Our file Notre référence
ISBN: 978-0-494-25088-4

Short title:

FTIR ANALYSIS OF EDIBLE OILS FOR FREE FATTY ACIDS AND MOISTURE

ABSTRACT

Three new quantitative Fourier transform infrared (FTIR) spectroscopic methods were developed for the analysis of edible oils: two procedures to measure free fatty acids (FFA) and one to measure moisture (H_2O), the latter two methods ultimately being automated and implemented on an auto-sampler equipped FTIR spectrometer. The methods developed for FFA determination both convert FFAs to their carboxylate salts by means of acid/base reaction without causing oil saponification, one approach using 1-propanol, an oil-miscible solvent, and the other using methanol, an oil-immiscible solvent into which the FFA salts are extracted. The first method involves splitting oil samples into two halves, with one half treated with propanol containing base and the other half with propanol only. The spectra of each half is collected and a differential spectrum obtained, from which quantization is performed. The methanol procedure simply involves extracting FFA into methanol containing a weak base and quantitating the FFA salts produced. Both FFA methods determine the FFA content by measuring the ν (COO^-) absorbance at ~1570 cm^{-1} relative to a reference wavelength of 1820 cm^{-1} from a differential spectrum relative to the solvent, the extraction procedure being superior in terms of both speed and sensitivity, being able to measure FFA levels down to ~0.001%. The method developed for moisture determination involves extracting water in edible oils into dry acetonitrile and then quantitating it by measuring the absorbance of the OH stretching band (3629 cm^{-1}) and/or the HOH bending band (1631 cm^{-1}). All three methods were validated by standard addition experiments, evaluated for potential interferences, and, in the case of FFA determination, compared to the performance of AOCS official methods. The results indicated that the extraction-based procedures were superior to conventional wet chemical methods in both sensitivity and reproducibility. The FFA and H_2O extraction procedures were subsequently automated by connecting an auto-sampler to the FTIR spectrometer and developing procedures and software algorithms to enable the analysis of up to 100 samples/h. The methods developed and implemented are a substantive improvement over conventional methods for the analysis of FFA and H_2O in edible oils and provide a means by which QC and process laboratories can analyze large volumes of edible oils for these two important parameters.

RESUME

Trois nouvelles méthodes d'analyse quantitative utilisant la spectroscopie à infrarouge à transformation de Fourier (FTIR) ont été développées afin d'analyser les huiles comestibles; deux méthodes pour mesurer les acides gras libres (AGL) et une méthode pour mesurer l'humidité (H_2O), ces méthodes ont été enfin automatisées et mises en application sur un spectromètre FTIR équipé d'un auto échantillonneur.

Les deux méthodes développées pour la détermination des AGL transforment les AGL en leur sels de carboxylate par le moyen de la réaction acide/base sans causer de saponification à l'huile, la première méthode utilise le propanol, un solvant miscible avec l'huile alors que la deuxième méthode utilise le méthanol, un solvant immiscible avec l'huile et dans lequel les sels des AGL sont extraits. La première méthode fait appel à la division des échantillons d'huile en deux moitiés, une moitié est traitée par une base dissous dans le propanol et l'autre moitié est traitée avec du propanol uniquement. Le spectre de chaque moitié est enregistré et un spectre différentiel est obtenu permettant ainsi la quantification. La méthode utilisant le méthanol fait simplement appel à l'extraction des AGL dans une solution de méthanol contenant une base faible et à la quantification des sels des AGL ainsi produits. Les deux méthodes déterminent le contenu en AGL en mesurant l'absorbance du ν (COO^-) à 1570 cm^{-1} respectif à une longueur d'onde de référence de 1820 cm^{-1} à partir d'un spectre différentiel respectif au solvant, elles sont capables de mesurer des niveaux des AGL aussi bas que ~0.001%. Une méthode pour la détermination de l'humidité a éte développée en extrayant l'eau dans les huiles comestibles dans de l'acetonitrile sec et le quantifiant par la suite en mesurant l'absorbance de la bande d'élongation OH (3629 cm^{-1}) et/ou de la bande de déformation HOH (1631 cm^{-1}). Les trois méthodes furent ensuite validées grâce à l'utilisation de techniques d'addition standard, evaluées pour les interférences potentielles et dans les cas de la détermination des AGL comparées à la performance des méthodes officielles de l'AOCS. Les résultats ont montré que les procédures basées sur l'extraction étaient supérieures aux méthodes chimiques conventionnelles en termes de sensibilité et reproductibilité. Les méthodes d'extraction des AGL et du H_2O furent ensuite automatisées en connectant un auto-echantillonneur au spectromètre FTIR et en développant des procédures et des algorithmes pour logiciels qui facilitent l'analyse jusqu'à 100 echantillons/h. Les méthodes développées et mises en application constituent une amélioration considérable par rapport aux méthodes conventionnelles d'analyse des AGL et H_2O dans les huiles comestibles et fournissent un moyen par lequel le CQ et les laboratoires des processus peuvent analyser de larges volumes d'huiles comestibles pour ces deux importants paramètres.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to **Professor F. R. van de Voort**, my supervisor, for the time, guidance and encouragement that he has given me throughout my PhD program; his exceptional qualities as a research supervisor have helped make this thesis possible. I wish to acknowledge my sincere gratitude to **Dr. Jacqueline Sedman** for her collaboration, valued advice and input and to thank **Drs. William Marshall, Selim Kermasha, Varoujan Yaylayan** and **Ashraf Ismail** for their guidance, advice and use of analytical instrumentation.

Very special thanks to **Sensei John Kalaidopoulos**, my senior karate teacher, who taught me *in practice* the true meaning of karate, for his support and understanding. Very warm thanks go to **Sempai Stephen**, **Sempai Tony**, my best friend **Denies** and all the karate students at the **West Island Kyokushin Karate** for their kindness, friendship and continuous help. I also wish to extend my warm thanks to my colleagues at the Food Science and Agricultural Chemistry Department and to my friends in Sainte Anne de Bellevue.

Above all, my deepest gratitude goes to ***my parents, family and my beloved woman*** for their continuous love, support and encouragement, with my personal thanks to Sultan Qaboos University (SQU) and Embassy of Sultanate of Oman, Washington DC, for the financial and academic support that they given me throughout my study in Canada.

CONTRIBUTIONS OF AUTHORS

Chapters 3-6 of this thesis comprise of the text of published or submitted papers listed below. The author of this thesis was responsible for the concept, design of experiments, experimental work, and manuscript preparation. Dr. van de Voort is thesis supervisor and had direct advisory input as the work as it progressed. Dr. Sedman provided advice relative to spectral interpretation, chemometrics and editorial assistance in the final stages of paper submission. Mr. Ghetler was responsible for the UMPIRE software platform on which FFA and H_2O algorithms were developed for the automated methods discussed in Chapter 6.

List of the publications reported in the thesis:

Al-Alawi, A., van de Voort, F. R. and Sedman, J. New FTIR Method for the Determination of Free Fatty Acids in Oils. *J. Am. Oil Chem. Soc.*, 81(5), 441-446 (2004).

Al-Alawi, A., van de Voort, F. R. and Sedman, J. A New FTIR Method for the Analysis of Low Levels of FFA in Refined Edible Oils. *Spectrosc. Lett.* 38(4-5) 389-403 (2005).

Al-Alawi, A., van de Voort, F. R. and Sedman, J. A New FTIR Method for the Determination of Low Levels of Moisture in Edible Oils. *Appl. Spectrosc.*, 59(10), 1295-1299 (2005).

Al-Alawi, A., van de Voort, F. R., Sedman, J. and Ghetler, A., Automated FTIR Analysis of Free Fatty Acids and Water in Edible Oils. *Journal of the Association for Laboratory Automation. (in press).*

CONTRIBUTIONS TO KNOWLEDGE

1. **Designed new analytical approaches for edible oil analysis to provide the industry with practical and readily implemented methodology.**

   New analytical strategies were developed to compensate for matrix effects arising in FTIR analysis of edible oils that are not readily modeled by chemometrics, thereby enhancing analytical accuracy and sensitivity, to facilitate sample handling, and to allow automation of the analysis.

2. **Developed the first quantitative FTIR method for FFA analysis in edible oil that avoids saponification and is independent of oil type.**

   Quantification was successfully achieved through the use of carefully selected weak bases which converted FFA to their salts without causing saponification of the oil. Generalization of the FTIR method so that matrix effects were eliminated and the spectral response was independent of oil type was achieved via differential spectroscopy.

3. **Developed a sensitive and simple method for determination of low levels of FFA in refined edible oils.**

   The sensitivity of the FFA method was extended by treating oils with an oil-immiscible solvent containing a weak base to convert the FFA to their carboxylate salts and concentrate the salts in a small volume. This procedure greatly enhanced the sensitivity of the FTIR FFA analysis as well as eliminating the requirement to prepare two samples to obtain one analytical result as in the first method devised.

4. **Developed a sensitive, simple and accurate method for the quantitative determination of moisture content in edible oils.**

   An FTIR-based instrumental method for the determination of moisture was developed, modeled on the extraction procedure developed for low-FFA determination, but without any requirement to carry out a stoichiometric reaction. The method eliminates most of the analytical problems that are commonly associated with moisture quantification in edible oils and provides a viable alternative to the widely used Karl Fischer method.

5. **Automated the extraction-based FFA and moisture analysis methods to carry out high-speed, high-volume analyses of these measures for QC and process laboratory purposes.**

The extraction-based FFA and moisture methods developed were refined and implemented to operate on an FTIR integrated with an auto-sampler and micro-pump to allow for automation of these methods. The system was developed, programmed, optimized and validated and was shown to be able to analyze up to 100 samples/h.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

1-PrOH	*n*-Propanol
AN	Acid number
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflectance
BR	Blank reagent
DMP	Dimethoxypropane
DMSO	Dimethyl sulfoxide
DTGS	Deuterated triglycine sulfate
EDTA	Ethylenediaminetetraacetic acid
FFA	Free fatty acid
FIA	Flow injection system
FTIR	Fourier transform infrared spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
K-Phthal	Potassium phthalmide
LIMS	Laboratory information management system
MD	Mean difference
MeOH	Methanol
MLR	Multiple linear regression
NIR	Near infrared
NSERC	Natural Sciences and Engineering Research Council
OD	Optical density
PLS	Partial least-squares regression
ppm	Parts per million
PV	Peroxide value
QC	Quality control
RBD	Refined, bleached and deodorized oil
ROOH	Hydroperoxide
RR	Reactive reagent

SD	Standard deviation
SDD	Standard deviation of the differences
SEFIA	Solvent extraction flow injection system
TAG	Triacylglycerol
TEA	Triethanolamine
TPP	Triphenylphosphine
TPPO	Triphenylphosphine oxide
UMPIRE®	Universal Method Platform for InfraRed Evaluation
w/v	weight per volume
w/w	weight per weight
ZnSe	Zinc selenide
Z-reg	Regression through the origin

CHAPTER 1

GENERAL INTRODUCTION

1.1. CONTEXT OF THE RESEARCH

Fats and oils represent a major and essential portion of our diet, and the fats and oils industry is one of the largest sectors of the food industry. According to *Euromonitor International,* consumer expenditure for fats and oils in 2005 was US $ 122.6 billion **(1),** comprising ~ 0.5% of the total consumer expenditures; this amount does not include by-products such as soap and meal cake. Because fats and oils are valuable and economically important food commodities, a variety of professional organizations have set standards for the edible oil industry in relation to determining their quality, functionality and the parameters affecting their economic value. Several prominent organizations vie to play official roles in this regard, including the American Oil Chemists' Society (AOCS) **(2),** the Association of Official Analytical Chemists (AOAC) and the International Union of Pure and Applied Chemistry (IUPAC) **(3).** Methods and standards adopted by these organizations are considered to be "official" methods, most being similar, albeit not identical in their detail. A wide range of official analytical methods exist for the routine evaluation and characterization of fats and oils, examples being iodine value (degree of unsaturation), saponification value (average molecular weight), moisture content, peroxide value, anisidine value and free fatty acid (FFA) content **(4),** to name just a few.

Most of the official procedures noted above are titrimetric methods developed in the 1930-40's using color indicators and many are still in routine use today. All are workable and are standardized; however, the lack of skilled labor and the safety/environmental issues associated with toxic solvents and reagents make these methods problematic in modern times. Apart from these drawbacks, most of these methods are mediocre in terms of accuracy, reproducibility and analytical range relative to newer instrumental methods. The poor accuracy is often associated with their non-specificity, the reliance on accurate color perception by the analyst or problematic dark samples, the latter obscuring the end point. **Table 1.1** shows an example of an inter-laboratory study of FFA and moisture results obtained for the same sample of cottonseed oil **(5)** as reported by 10 different certified laboratories using the same official methods.

Table 1.1. Data Entry Report for Cottonseed Oil (2001-2002). Adopted from ref. **(5)**

Analyst	FFA[a]	Moisture[b]
1	0.14	0.10
2	0.15	0.05
3	0.16	0.09
4	0.15	0.10
5	------	0.07
6	0.04	0.09
7	0.17	0.04
8	0.05	0.08
9	0.18	0.06

[a] % w/w expressed as oleic acid; [b] % w/w

Such variations can increase even further as a result of biases which may interpose themselves when "official" methods from different organizations are used (e.g. AOCS *vs.* IUPAC), as each has different procedure. For environmental, sample throughput and accuracy reasons, there has been an ongoing effort to develop new instrumental approaches to titrimetric based analyses, focusing on automated titrators and continuous flow injection systems using various potentiometric and colorimetric detection systems.

An alternative to titrimetric analyses is the application of spectroscopic techniques to determine oil quality parameters based on spectral characteristics of the sample. In particular, a wide range of oil parameters can now be analyzed by FTIR spectroscopy **(6)**, providing alternative approaches to their assessment. The McGill IR Group has been at the forefront of the development of instrumental methods for the analysis of edible oils based on Fourier transform infrared (FTIR) spectroscopy. Whereas infrared spectroscopy was generally regarded in the past as a qualitative technique, providing functional group information, it has now evolved into a quantitative spectroscopic procedure, supported by

powerful chemometrics. Thus quantitative work with FTIR instrumentation can now approach the simplicity of using UV/visible spectroscopy, while providing much more specific spectral information in relation to the molecular species targeted for analysis. However, for complex samples such as edible oils there are certain inherent limitations to FTIR analysis such as matrix effects, overlapping bands, hydrogen bonding effects, etc. Chemometric techniques such as partial least squares regression can overcome some of these issues, but not all of them. Method development by the McGill IR Group has undergone an analytical evolution which exploits a range of strategies to obtain ever better sensitivity, accuracy or speed. The ultimate goals are to improve sensitivity and accuracy and ultimately to standardize and automate edible oil analysis by FTIR spectroscopy.

1.2. RATIONALE OF THE WORK

Of the variety of methods one could tackle with the above goals in mind, FFA analysis was my personal choice, based on the thousands of titrations which I had the misfortune to carry out in an oil processing plant, being a good motivator. Although FTIR methods for FFA determination have been previously developed, they suffer from several shortcomings. Similarly, there are well known problems associated with the titrimetric determination of moisture by the Karl Fischer method and this provided the impetus for developing methodology for the determination of moisture in edible oils by FTIR spectroscopy. Both FFA and moisture analyses are used to control many steps in the overall oil refining process, such as deodorizer efficiency and soap adsorption, and to evaluate storage conditions and oil stability. Thus these analyses determine important quality and process control parameters which need to be determined accurately and precisely. An examination of the literature regarding the current status of FFA and moisture analysis, indicates that there is still substantial room for improvement in terms of accuracy, specificity and practicality as well as a need for methods that can be automated by QC and process laboratories. Chapter 2 of this thesis reviews and details the relevant analytical methodology and approaches to the analysis of FFA and moisture to provide a context for the FTIR methods developed. In Chapter 3, a method for the determination of FFA based on a stoichiometric reaction coupled with differential spectroscopy is presented. Chapter 4 revisits the method, considering extraction as a

means of increasing sensitivity. Chapter 5 describes a method developed for moisture analysis drawing in part on the successful approach used in Chapter 4 for FFA. Chapter 6 takes latter two methods and adapts and implements them on an auto-sampler equipped FTIR to automate the methods attaining analysis rates of ~100 samples per hour as well as testing them to determine their overall performance. Each chapter is presented as a paper, Chapters 3-5 having been published, with Chapter 6 in preparation for submission. It has been a challenge and learning experience to develop methodology which I personally feel will eventually impact the edible oil industry in a tangible way in the future.

1.3. REFERENCES

1. Consumer expenditure on oils and fats: National statistical offices/OECD/Eurostat/Euromonitor International
2. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, IL, 1998.
3. IUPAC, *Standard Methods for the Analysis of Oils, Fats and Derivatives,* 7th edn., Blackwell Scientific, London, 1992.
4. O'Brien, R. D., *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing Company, Lancaster, PA, 1998.
5. Laboratory Proficiency Program, Cottonseed oil, AOCS, Champaign, IL, 2002.
6. van de Voort, F. R., Sedman, J. and Russin, T. Lipid Analysis by Vibrational Spectroscopy, *Eur. J. Lipid Sci. Technol.* 103, 815-826 (2001).

CHAPTER 2

LITERATURE REVIEW

2.1. FREE FATTY ACIDS (FFA) ANALYSIS

2.1.1. Introduction

Free fatty acids (FFA) are common triglyceride hydrolysis products found in crude oils and to some extent in refined oils as well as developing as a result of oxidation or frying, ultimately impairing oil quality and functionality. Chemically, FFAs are less stable than triglycerides and therefore more likely to oxidize and cause rancidity **(1)**. From a quality standpoint, the FFA content of edible oils is not only a crucial factor associated with their quality and economic value, but also an important quality indicator in relation to fats and oils processing, i.e., serving as a measure of deodorizer efficiency **(1)**. The AOAC, AOCS, and IUPAC all have standard methods for the determination of FFA, with the AOAC and AOCS methods being identical. These methods are based on the titration of an oil (3.5-56.4 g) dissolved in hot neutralized 95% ethanol (50-100 ml) **(2)** or ethanol/diethyl ether (50-150 ml) **(3)** with a strong base to a phenolphthalein end point under vigorous shaking. Although simple, these titrimetric methods are tedious and consume substantial amounts of solvent which are costly and difficult to dispose of safely; in addition, the use of a colorimetric indicator is problematic when dark crude oils are analyzed.

In recent years, a variety of approaches have been investigated as possible alternatives to the manual titrimetric methods employed to determine the FFA content of oils. These generally require more sophisticated equipment such as flow injection systems, automatic titrators and chromatographic instrumentation, among others. Many of these techniques offer some benefits in terms of speed of analysis, amenability to automation, and/or a reduction in the use of solvents, with the attendant environmental benefits, and some provide a substantive gain in sensitivity over that attained with titrimetric methods. The objective of this section is to review in general terms a variety of approaches to FFA analysis and place the development of FTIR methodology in context.

2.1.2. Historical Perspective

After World War II, the food industry began a new stage of development and expansion to catch up with the increased need for food as a result of the growing population. As an integral part in the food chain, the oil industry expanded dramatically which led to revision/re-evaluation of many quality associated parameters and the methods that are used to determine them. FFA determination was among the important methods that were subjected to many studies and modifications to make it more sensitive and reproducible as well as more rapid and economical. Over the past sixty years, many methods were published, and each method utilized the analytical technology advances associated with its time to improve the method as well as addressing the health and environmental issues of concern at the time of publication. For example, as spectrophotometry was the most well known analytical technique in the 1950s, most of the FFA methods published at that time (and later) were colorimetric methods as opposed to the previous generation of methods which were characterized by the use of manual titration for quantification. Later on, the methods tended more towards electrochemical techniques.

2.1.3. Colorimetric Methods

Copper soap method. The first attempt to develop a colorimetric method for FFA was carried out by Ayers in 1956 **(4)**. FFAs were converted into water-insoluble soaps by reacting them with an aqueous solution of copper nitrate or cobalt nitrate. Quantification was carried out by extracting the colored soap precipitate (blue for copper and pink for cobalt) in chloroform followed by measurement of absorbencies at 675 nm for copper and 527 nm for cobalt. Although the method was not intended for FFA analysis per se, it provided a basis for other researchers to develop colorimetric methods for FFA analysis in edible oils.

In 1964, Baker **(5)** introduced his improved version of the cupric method which was designed especially for FFA analysis in edible oils. The method involves dissolving the oil sample (8 g) in benzene (50 mL) followed by a mixing step where an aliquot (10 mL) of the mixture is shaken with aqueous cupric acetate solution in a separate tube. The mixture is centrifuged or let settle until a clear separation between the two phases is

obtained. Quantification of FFA is determined by measuring absorption of the upper benzene layer at 640 nm. A schematic diagram of the procedure is shown in **Figure 2.1**. Although Baker claimed a good correlation between his method and the titrimetric method, he indicated that certain copper soaps such as copper salts of palmitic and stearic acids do not dissolve in benzene. Discrepancies between the parameters used by Barker and Ayers, such as the working wavelength (640 *vs.* 675 nm), led Bains *et al.* **(6)** in the same year to examine the spectra and the effect of oil color on the measurement. The author concluded that 670 nm is the optimum wavelength, giving high sensitivity for quantification of copper soaps in colored or non-colored oil.

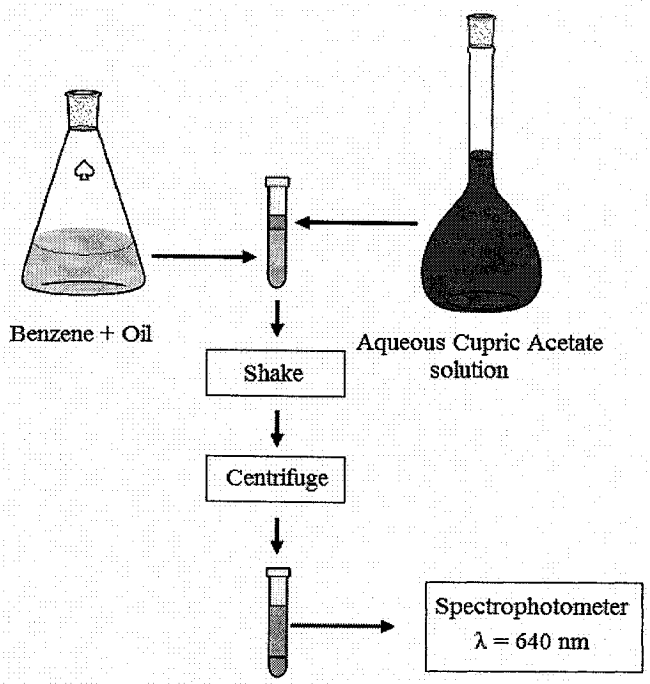


Figure 2.1. Schematic diagram of the copper soap method for FFA analysis introduced by Baker in 1964 **(5)**.

Despite the simplicity of the method, not much work was done to understand the chemistry of the products formed or even to optimize the conditions of the method to make it more sensitive and reproducible. In 1976, Lowry and Tinsley **(7)** published a considerable work about the conditions and chemistry involved in order to provide a basis to optimize the method and make it more sensitive and reproducible. The outcome of

their work was a stabilization of the copper soaps of saturated FFA in benzene as well as an increase in the sensitivity of the measurement by a factor of 10 just by controlling pH by adding pyridine. Due to both health and practical concerns, a number of publications came out suggesting a replacement for benzene, including toluene **(8)**, isooctane **(9)** and cyclohexane **(10)**.

In 1981, Ekstrom and Rhee **(8)** introduced the first automated system for FFA analyses in edible oils using the copper soap method which was able to analyze up to 20 samples/h. Automation was achieved using a flow injection analysis (FIA) system to mix oil samples with the solvents and reagents used in the method. According to the setup of the system, separation of the organic phase from the aqueous one was achieved online through the use of segmentors and separators at specified locations in the system. In contrast with the previous versions of the method, the measurement was done in the aqueous phase instead of the organic phase. This was possible by implementing two mixing-separation steps in the system; the first one was responsible for mixing the oil-solvent mixture and cupric solution together to produce FFA-copper soaps and then separate the two phases from each other (**Figure 2.2**).

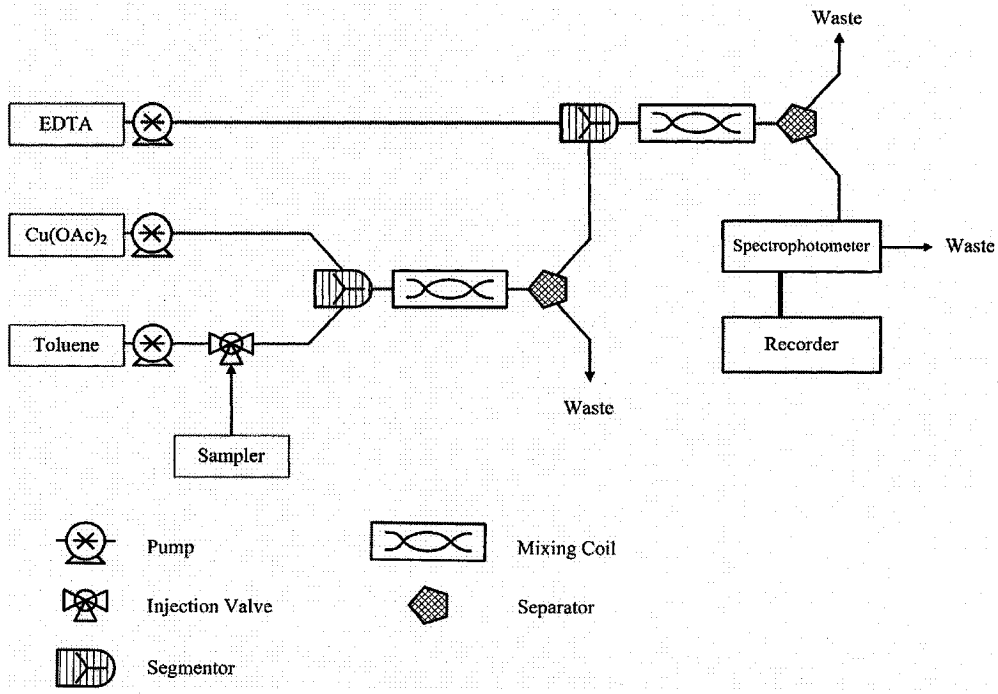


Figure 2.2. Flow diagram of the FIA instrument for determination of FFA in oil samples as proposed by Ekstrom and Rhee **(8)**.

The second step involved extracting the copper ions in FFA-copper soaps into another aqueous solution containing EDTA; and then the copper-EDTA complex formed was measured spectrophotometrically at 660 nm. The system did not receive much attention, mainly because it was complicated and had stability problems. Six years later, Canham and Pacey **(11)** reported an improved FIA system using a one-extraction-step process and improved hardware (segmenter and separator) illustrated in **Figure 2.3**. The new system had improved stability and shorter analysis time (130 samples/h), but at the expense of sensitivity. The sensitivity of FIA systems was boosted by Puchades *et al.* in 1994 **(12)** mainly by optimizing operating conditions, such as flow rate, pH, tubing length/diameter, and oil/solvent proportion, while maintaining good analysis speed (up to 75 samples/h). Two years later, Zhi *et al.* **(13)** came up with a new solvent extraction FIA (SEFIA) setup and protocol for FFA analysis without requiring a segmenter or separator. The method had the same high sensitivity as the previous method, but the analysis time was significantly increased (12 samples/h) and the FIA system required substantial coordination and timing which was provided by a minicomputer.

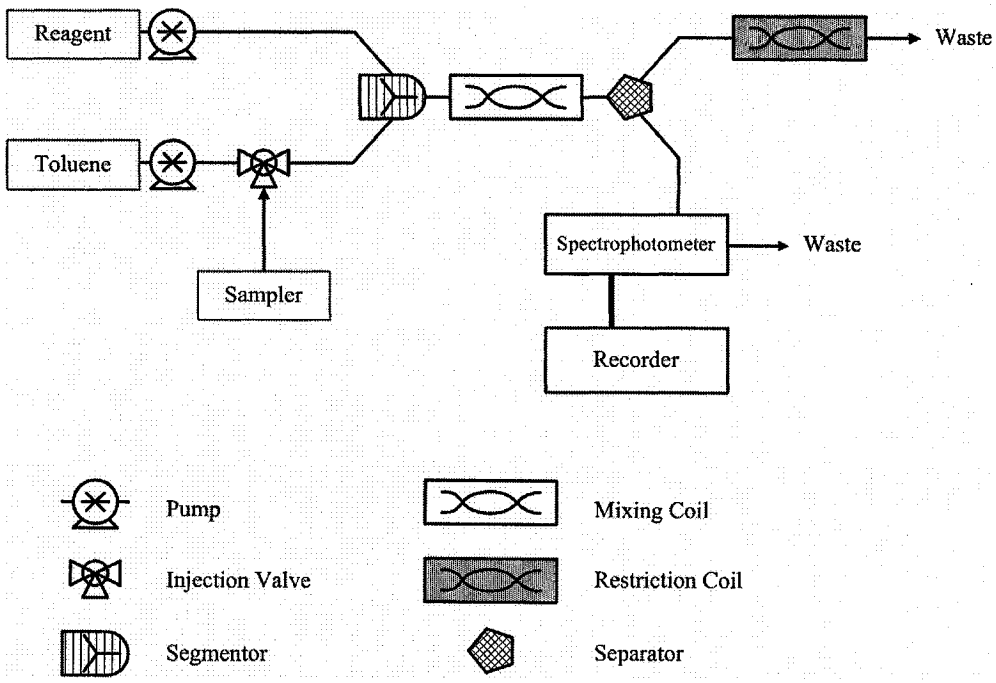


Figure 2.3. Flow diagram of the FIA instrument for determination of FFA in oil samples as proposed by Canham **(11).**

Despite the advantages offered by the copper soap method (limit of detection ~0.01 %FFA) and its automated versions (~12-130 samples/h), the method did not receive much attention from the oil industry, mainly because of the complexity of the analysis compared to the official method. Moreover, the FIA systems employed in the analysis had specialized hardware (segmenters and separators) which made them not suitable for other analyses.

Phenolphthalein based method. Despite the drawbacks of the "official" method, its simplicity and wide recognition made its automation a necessity in order to shorten the analysis time and reduce the ambiguity in determining the end point. The first substantive attempt to automate the official method was carried out by Linarers *et al.* in 1989 **(14)**. The setup of the system was simple (**Figure 2.4**) and used the same reagents as the IUPAC official method **(3)** whilst quantification was based on monitoring the decrease in the phenolphthalein intensity (at 562 nm) as a result of the reaction between KOH and FFA in the oil sample. The system had an analytical range of 0.15-0.81 % FFA and sample throughput of about 60 samples/h. In 1997, Nouros *et al.* **(15)** reported a modification with a similar analytical range, the mixing coil being replaced with a mixing chamber plus using a less expensive and less problematic solvent (1-propanol) as a diluent and carrier. The sampling rate was reported as 30-100 samples/h, and solvent consumption as 3-7 ml per sample. A similar system equipped with a photo probe connected to a spectrophotometer was reported in 2001 by Mariotti and Mascini **(16)** for FFA measurement in virgin olive oil. The system had the same sensitivity as the previous ones with a sample throughput of 12-60 samples/h.

Phenol red with reverse micelles. In 1990, Walde **(17,18)** developed a new approach for FFA determination in non-aqueous medium without titration. The method relies on the acidity of FFA to cause color changes to the alkaline form of phenol red. The indicator, which is water soluble, is dispersed in the non-aqueous medium (oil/isooctane) by bis(2-ethylhexyl) sodium sulfosuccinate, a surfactant. Quantification was achieved through monitoring changes in absorbance at 560 nm (disappearance of the red color). Although this method was economical in oil and solvent usage (0.1 ml and 5 ml, respectively), it was found to be lacking in accuracy and no further attempts have been made to optimize or automate it.

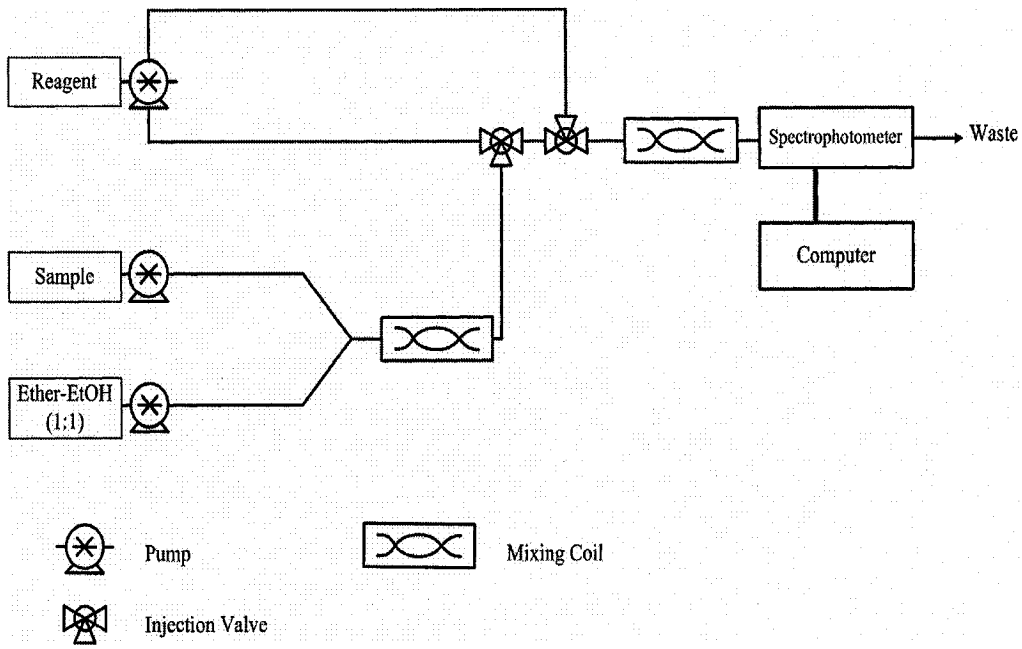


Figure 2.4. Flow diagram of the FIA instrument for determination of FFA in oil samples as proposed by Linarers *et al.* in 1989 **(14).**

2.1.4. Electrochemical Methods

Voltammetric techniques. Voltammetric techniques are widely used for compounds that are readily reduced or oxidized to provide highly selective and sensitive quantitative methods. Although FFA can be oxidized or reduced, they are by nature weakly active electrochemically, a factor which impeded the development of reliable electrochemical methods for FFA determination. The situation was changed in 1972 when Takamura *et al.* **(19)** published their work on the voltammetric behavior of quinone in the presence of acids in unbuffered protic solvents. It was reported that addition of acids to the reduced form of quinone (dissolved in ethanol) gave a very strong voltammetric signal. The behavior of quinone has been utilized by many authors **(20-25)** to develop FIA and HPLC methods for FFA analysis in edible oils. The FIA system coupled with voltammetric detection had high sensitivity/reproducibility (0.05 % FFA, RSD 0.7% **(23)**), low solvent consumption and outstanding throughput (60 samples/h)

pH-Metric methods. The pH meter obviously comes to mind as a means to measure acidity (FFA); however, the high viscosity and low polarity of oils have made it

impractical to use pH-metric methods. In 1991, Labshina *et al.* **(26)** suggested extraction of FFA from oil samples into a polar solvent to carry out pH measurements. In order to facilitate extraction and pH measurement, the authors suggested use of a weak base, triethanolamine (TEA), in a suitable solvent mixture (water (1%), diethyl ether (80%) and chloroform (19%)) to convert carboxylic acids into their salts. Although workable, the method had poor reproducibility, mainly because of instability of the electrode and the basic reagent in the solvent mixture (due to the low concentration of water). The method was later modified by Tur'yan *et al.* **(27)**, who replaced the former solvent mixture with a 1:1 (v:v) mixture of *iso*-propanol and water containing 0.2 M TEA and 0.02 M KNO_3, the latter added to obtain a constant ionic strength. Rather than extracting FFAs, this solvent mixture effectively makes an emulsion within which the pH measurement is made. Various versions of this approach were developed for acidity measurements in petroleum oils **(28)**, oilseeds **(29)** and hydraulic oils **(30)**. Although providing a simple means of measuring FFAs, it does not provide any improvement over the official titrimetric method in terms of sensitivity or solvent consumption.

Automated potentiometric titration. One of the main reasons that the official method is still in use is the advances that have been made in potentiometric measurements and automated titration systems. The first application of potentiometric measurements to determine the end point of acid/base reactions goes back to the early years of the last century. It was realized that a potentiometric end point is sharper and more reproducible in determining the end point of a reaction than using a color indicator; however, a lack of the technology required to automate the measurement limited the acceptance of this approach as a practical alternative to the conventional titration. Since World War II, substantial effort has been devoted to improving potentiometric response as well as automation and has resulted in the commercial availability of potentiometric auto-titration systems **(31-35)**. The basic setup of such a system consists of a potentiometric electrode (pH meter), syringe pump, and stirrer and a recorder (or computer). Initial automated titrators were large and had few features, but computerization has overcome many of their initial limitations, resulting in general acceptance in analytical labs. **Figure 2.5** presents a modern potentiometric auto-titration system which in brief, involves volumetric addition of the titrant (alkaline solution) from

the bottle at the rear (see **Figure 2.5**) in small increments by the syringe pump while the pH is measured by the electrodes immersed in the beaker of analyte on the stirrer. By convention, the titration curve has the volume of titrant added on the x-axis and the corresponding change in pH (expressed as pH units or mV) on the y-axis. The end point of the titration is determined as the midpoint of the maximum slope of the titration curve. Once the end point is detected, the volume of the titrant added stoichiometrically defines the concentration of FFA in the oil sample.

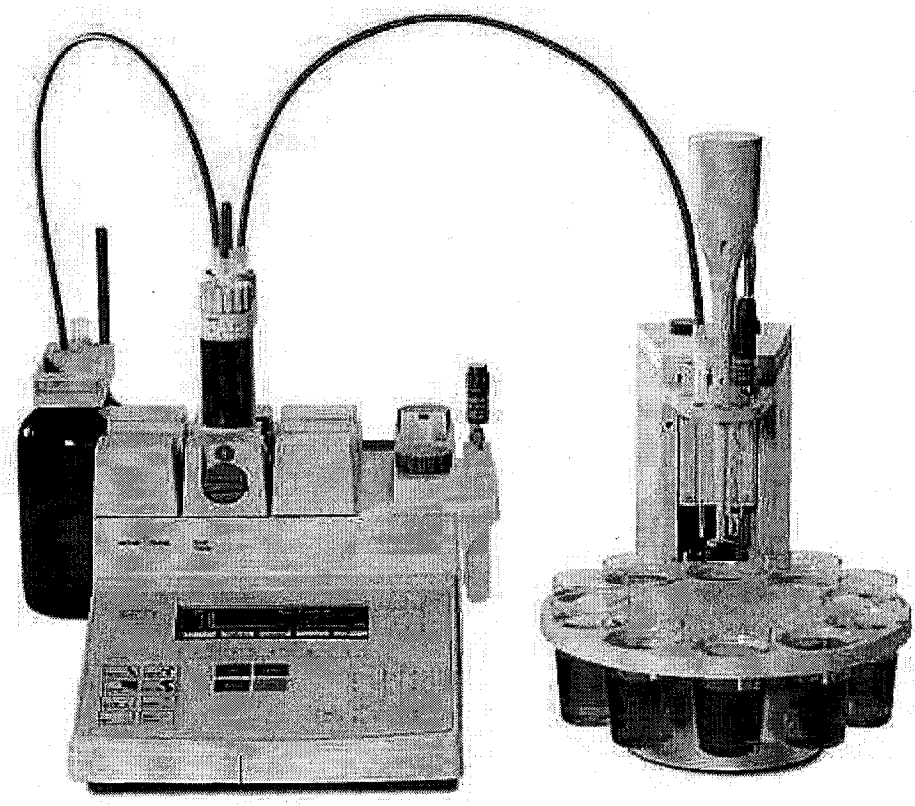


Figure 2.5. Metrohm auto-titrator instrument equipped with autosampler for FFA analysis.

Auto-titrators are available commercially from a number of suppliers, such as Metrohm, Mettler, Labcor, etc., and can be equipped with many optional accessories, e.g. autosampler, different sensors, etc. For FFA analysis, most of the titrators use glass electrodes to measure changes in pH, a 1:1 mixture of ether and ethanol as diluent, and

KOH/ethanol (or KOH/*tert*-butanol) **(36)** as titrant. The accuracy of the auto-titrator systems depends mainly on the capability of delivering small and consistent amounts of the titrant, which varies between different manufacturers. The sensitivity, on the other hand, depends more on the pH electrode; if the electrode receives proper conditioning treatments, good sensitivity (on the order of 0.01% FFA) will be maintained. Potentiometric auto-titrator apparatuses are the most widely used systems for FFA analysis in quality control labs.

2.1.5. Thermometric Methods

Thermometric methods are titrimetric methods that use the change of temperature resulting from a chemical reaction to determine the end point of the titration. The principle of this type of method is the same as that for potentiometric auto-titration; the only difference is the type of sensor (detector). The detectors (usually thermistors) used in this kind of method show large changes in their electrical resistance for small changes in temperature. The first considerable advance in determining the end point of the acid/base reaction in non-aqueous medium thermometrically dates back to the 1960s. In 1965, Vaugham **(37)** reported that upon titration of a sample containing a weak acid dissolved in acetone, the first excess of base catalyzes a strong exothermic reaction which can be detected easily. Since the introduction of this method, many modifications have been made which involve the use of different reagents and solvents in order to make the method more sensitive and the end point sharper. In 2003 Thomas **(38)** reported that acetone and chloroform react exothermically (and violently under certain conditions) in presence of base, and performance experiments using a commercial automated thermometric titration unit with KOH in *iso*-propanol as titrant showed exceptional reproducibility in determination of FFA in edible oils. Titrations using thermistor detectors are becoming popular in quality control labs, because these sensors are largely maintenance-free.

2.1.6. Current Status of FFA Analysis

Many of the instrumental methods discussed above offer substantial benefits over traditional manual titration in terms of speed of analysis, amenability to automation, and/or a reduction in the use of solvents or amelioration of disposal costs. However, the

colorimetric methods have inadequate sensitivity, with the additional drawback of requiring frequent calibration, which makes them generally impractical for routine analysis. Electrochemical methods require frequent titrant standardization, and the high accuracy and reproducibility that these methods can provide are also dependent on the conditions of the electrodes, making regular maintenance necessary. Moreover, none of the methods discussed provide specificity to FFA measurement as they tend to be based on measures of total acidity rather than FFA per se. Thus, there remains a need for a specific, accurate and simple method for the analysis of FFA in edible oils.

2.1.7. Infrared Spectroscopic Methods

The possibility of employing FTIR spectroscopy in FFA analysis has been investigated by a number of researchers over the past fifteen years. The first such study, reported by Lanser *et al.* **(39)** in 1991, was directed toward the development of a semi-quantitative method for the analysis of soybean oils containing appreciable levels of FFA. The FTIR spectra of oils placed between two KBr windows were collected, and the spectral changes observed in the carbonyl region, after deconvolution of the spectra over the 2000-1600 cm^{-1} range, were correlated to the FFA content obtained by the standard AOCS method. The method was placed on a quantitative basis by devising a calibration using standards prepared by spiking oleic acid into FFA-free soybean oil. It was noted that because the carboxylic acid C=O absorptions appear as a broad shoulder on the strong triglyceride ester linkage absorption, a major limitation to making the method more quantitative was the spectral variability of the ester band among different oils. In 1993, Ismail *et al.* **(40)** developed a more rigorous quantitative method based on measuring the carboxylic acid C=O band at 1711 cm^{-1} after applying oil samples in their neat form onto an attenuated total reflectance (ATR) cell. The matrix effects noted by Lanser *et al.* **(39)** were reduced by ratioing the calibration and sample spectra against the spectrum of an FFA-free oil of the same type as the oil being analyzed, rather than an air background spectrum. In recognition of the limited utility of this approach in eliminating matrix effects in the case of crude and oxidized oils **(40)**, an alternative indirect method was also developed. This method involved adding KOH/MeOH to the oil so as to convert any FFAs present to their salts, effectively moving the measurement band from 1711 cm^{-1} to 1570 cm^{-1}, a region where there are no major interferences which limit the accuracy of

peak height measurements (see **Figure 2.6**). In addition to overcoming matrix effects, this indirect method was more sensitive than the direct measurement of FFA absorption, albeit at the expense of complicating the analysis. However, the method was susceptible to error due to saponification of the oil by the KOH/MeOH reagent.

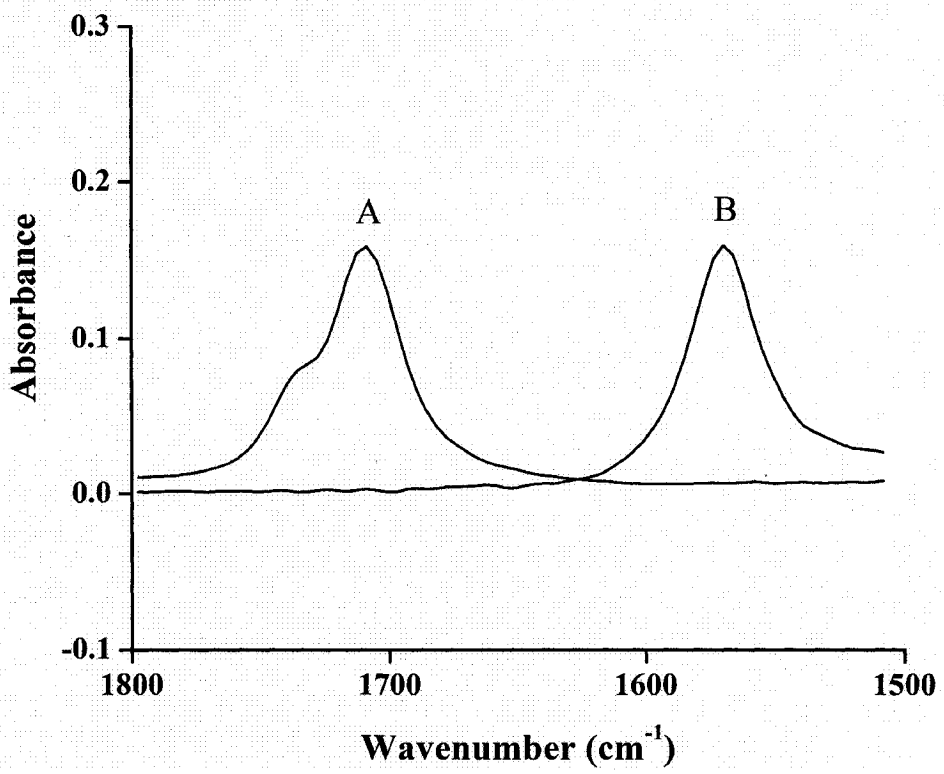


Figure 2.6. Shift in FFA band after conversion to carboxylate salt. FFA shows band at 1711 cm^{-1} (A); the band shifts to 1570 cm^{-1} after addition of base (B). (The spectra were taken in our lab)

After these initial studies, two publications dealt with FFA analysis by FTIR spectroscopy in relation to palm and olive oil, respectively **(41,42)**, both using partial least squares (PLS) to develop relationships between spectral changes and the results obtained with the standard method. Subsequently, Verleyen *et al.* **(43)** followed up more rigorously on the initial work of Lanser *et al* using peak height measurements at 1711 cm^{-1} to develop workable calibrations for a variety of oils but ultimately concluded that the calibrations were heavily oil dependent.

The most sophisticated work related to FFA analysis has been that of Cañada *et al.* **(44)**, who developed an elegant automated FTIR-based continuous-flow analysis

system capable of analyzing ~40 samples/h. For this system, these authors adapted the indirect KOH/MeOH method developed by Ismail *et al.* **(40)** as an effective means of overcoming the matrix effects associated with direct measurement of the carboxylic acid C=O band. Having noted the susceptibility of oils to saponification by KOH even within the 30-s mixing time employed, Cañada *et al.* modified the system to eliminate this source of error by reducing the contact time between KOH and the oil (premixed with MeOH) to ~2 s.

Aside from these FTIR studies, FFA analysis by other types of vibrational spectroscopy, namely, near-infrared (NIR) and Raman spectroscopy, has also been investigated **(45-49)**. All the NIR and Raman methods that have been published to date are direct methods in the sense that there is no sample preparation involved and the FFA measurement is done using the spectrum of the neat oil. In addition, all these methods use PLS and/or multiple linear regression (MLR) calibrations that have been developed for specific oil types and hence cannot be applied to other oil types or samples of unknown origin. Moreover, the NIR and Raman methods, although having the benefit or being reagent-free, tend to have relatively low sensitivity ($\geq$ 0.3%), which makes them suitable largely for the analysis of oils known to contain high amounts of FFA such as fish **(45,46)**, palm **(47)**, and olive oils **(48, 49)**.

2.2. MOISTURE CONTENT ANALYSIS

2.2.1. Introduction

Moisture content in fats and oils is considered a key quality parameter in the edible oil industry and has to be controlled throughout oil processing, this being especially important when oils are being pressed from high-moisture raw materials. Moreover, water is commonly added to oils during degumming and refining operations which then needs to be removed, usually by centrifugation and/or vacuum drying. Attaining a low moisture content after refining is very important for the effective adsorption and removal of traces of soap in the oil **(2)**.

Water has limited solubility in oils and fats, ranging from 0.05 to 0.3% **(50)**; however, from a quality standpoint, the moisture content should be reduced to the lowest practical level. By convention, moisture content of dried refined oil should be below 0.1% and is most often on the order of 0.05% **(1)**. Hydrolysis, breakdown of fat, is induced by the presence of moisture, accelerated by heat and/or residual enzymes, and results in the formation of FFA, di- and monoglycerides and glycerol. Hydrolysis can result in off-flavors, a reduced smoke point, increased fat absorption and darkening of shortenings used for frying **(1)**. Maintaining a low moisture content will limit hydrolysis of triglycerides during processing and storage **(1)** and avoid the formation of FFA, which are susceptible to auto-oxidation. Thus, the routine determination of moisture would be helpful in ensuring acceptable oil quality; however, moisture analysis in oils is both difficult and problematic.

There are many classic methods for the determination of moisture in edible oils and related products, but they can be grouped into three categories: evaporation, distillation and titration procedures. The *Official Methods of the American Oil Chemists' Society* **(51)** lists several evaporation procedures which involve heating oil samples on a hot plate (Ca 2b-38), in an oven (Ca 2c-25), or in a vacuum oven (Ca 2d-25). Although these procedures are simple, there are many problems associated with them. For example, the evaporation of moisture may also remove other volatile components, such as short-chain FFAs, and as a result the weight loss recorded is not only due to loss of moisture. Highly unsaturated oils can oxidize during the evaporation process and an increase in weight may actually be recorded, while triglycerides may undergo hydrolysis by the moisture initially present giving rise to FFAs.

The distillation approach is based on the *azeotropic* property of water which is the ability of water to form constant boiling mixtures with many organic solvents in which it is immiscible when cold. The AOCS distillation method (Ca 2a-45) involves mixing 20-200 g of the test sample with 100–300 ml of toluene. The mixture is heated and water is distilled out of the sample as a constant boiling mixture with the solvent. The distillate separates with water forming the lower phase, whereupon its volume is measured in a specially graduated tube. Although it is an accurate procedure per se, the distillation method has many drawbacks that limit its use, such as its limited sensitivity (not

applicable to samples containing less than 0.5% moisture), the large sample size required, and its lack of suitability for high-volume testing.

The "best" method available for moisture determination is the Karl Fischer titrimetric method (e.g., AOCS Ca 2e-84), and it is the gold standard against which proposed new methods for moisture analysis are compared. As such, this method will be discussed in detail, and proposed modifications that have been reported in the literature will be surveyed to provide a clear picture of its current status.

2.2.2. Karl Fischer Method

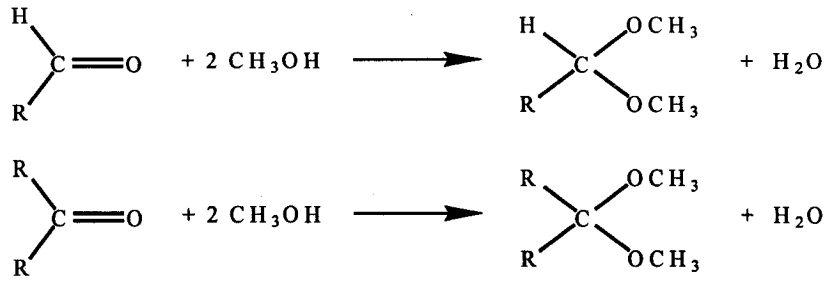
The Karl Fischer (KF) method involves the chemical reaction of water with the Karl Fischer reagent (mixture of iodine, sulfur dioxide, pyridine (or similar base) and methanol), which consumes water stoichiometrically. The general reaction is as follows:

$$SO_2 + CH_3OH + B \rightleftharpoons CH_3SO_3(HB)$$
$$I_2, I_3^- + H_2O + CH_3SO_3(HB) + 2B \longrightarrow CH_3SO_4(HB) + 2(HB)I \qquad [2.1]$$

where B is a base, such as pyridine or imidazole. The general protocol involves mixing 5–25 g of oil with anhydrous methanol (or 1-propanol) and titrating with Karl Fischer reagent to a cherry-red colored end point. Due to the high error associated with manual titration (0.6% relative error) **(51)**, the titration is frequently carried out automatically in a closed vessel where the end point is determined potentiometrically. The automated method can determine as low as 200 ppm of water. Although detection of the end point by potentiometric measurement is more sensitive than in the case of manual titration, it is still a titrimetric method and, as such, standardized reagents are required to obtain consistent and accurate results. The need for standardization is eliminated by using coulometry; an additional advantage of coulometric measurements is their higher sensitivity, which in the case of the KF reaction extended the limit of detection to cover the range 1-25,000 ppm water.

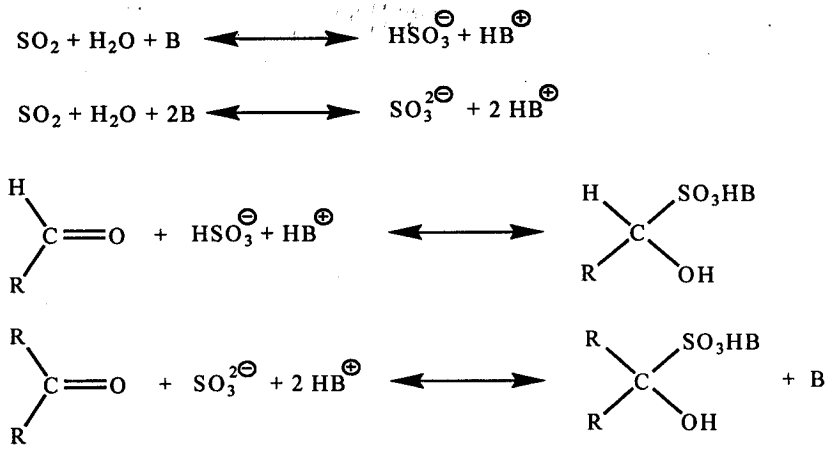
Although the KF reagent is specific to water, the presence of aldehydes and ketones can complicate the analysis by introducing side reactions. Two side reactions are

well known to take place during KF measurement which directly interfere with accurate moisture determination and are believed to occur "simultaneously" in the presence of aldehydes/ketones. The first reaction is acetal/ketal formation where methanol reacts with aldehydes/ketones to produce water molecules as shown in Eq. 2.2.



[2.2]

The occurrence of such reactions increases the number of water molecules in the system, leading to an overestimation of moisture content in the sample being analyzed. The second reaction is called bisulfite addition. Bislfite ions are common products in KF systems, being formed reversibly by the reaction between water molecules and sulfur dioxide under basic conditions (Eq. 2.3). In the presence of aldehydes/ketones, the bislfite ions undergo addition reactions and form bisulfite-addition products as shown in the reaction scheme below:



[2.3]

As can be noted from these reaction equations, these reversible reactions consume water, which is then released back into the system owing to the shifting of the equilibrium to the left as water reacts with the KF reagent. Accordingly, these reactions delay reaching of the end point, leading to an underestimation of the water content in the sample when these reactions are not taken into account in setting the instrument parameters.

The reaction conditions and rates of the above reactions were extensively studied by many authors in order to minimize the interference as much as possible. In this regard, Scholz **(52)** reported after studying the behavior of 44 different aldehydes and ketones with different KF formulations that acetal/ketal formation reactions are acid-catalyzed and that higher pH had a significant effect in reducing the extent of these side reactions. He suggested use of the stronger base imidazole as a substitute for pyridine in the KF formulation in order to make the reaction conditions more basic. Scholz **(52)** agreed with other authors who found that replacement of methanol with other solvents, such as 2-methoxyethanol **(53)**, dimethylformamide **(54)**, and propylene carbonate **(55)**, suppressed acetal/ketal formation reactions, but he found that the bisulfite addition reaction is more favored in nonalcoholic solvents; consequently, he suggested use of alcoholic solvents and higher pH rather than use of nonalcoholic solvents as a means of suppressing side reactions of aldehydes and ketones with KF reagents. Examination of different types of alcoholic solvents (primary, secondary and tertiary alcohols) as an alternative to methanol led Scholz to strongly recommend that 2-chloroethanol and 2,2,2-trifluoroethanol be used in the KF formulation instead of methanol. He reported that KF formulations using either of these solvents with imidazole as the base had outstanding performance in determination of water in the presence of aldehydes and ketones; in the same year he patented a new KF reagent using those two solvents and imidazole **(56)**. In 1987, Andersson and Cedergren **(57)** reported that the bisulfite addition reaction is a slow reaction and, therefore, suggested use of fast-reacting KF reagents or higher iodine concentration to reduce the analysis time in order to make the onset of the bisulfite addition reaction negligible. In addition, the authors **(57)** found no observable improvements when 2,2,2-trifluoroethanol was used in place of 2-methoxyethanol. Bizot **(58)** and Cedergren **(59)** reported that addition of some chemicals such as formamide to

pyridine-buffered methanolic KF reagent was found to speed up the KF reaction by a factor of 100, which in turn can minimize the problems connected with slow reaction rates between KF reagents and water. Alternatively, Scholz **(60)** reported that replacement of pyridine with imidazole (more basic than pyridine) was found to increase the speed of the reaction of the KF reagent with water and ultimately minimize the effect of both the bisulfite addition reaction and acetal/ketal formation. In 1994, Orädd and Cedergren **(61)** showed that in order for imidazole to speed up the reaction the pH has to be in the range 7-10.

Besides the side reactions of water production/consumption due to presence of active carbonyl groups, there are other important side reactions associated with iodine. In coulometric KF analysis, iodine is formed at the anode and reacts quantitatively with water present in the system as shown in Eq. 2.1, with the end point of the titration being the point at which iodine is no longer consumed. Iodine is known to be a strong oxidizing agent and can react with substances such as thiosulfite, thiosulfate, ascorbic acid, hydrazines, hydroxylamines, Tl, Sn^{2+}, In^{+} and Cu^{+}, etc., in the matrix being analyzed, resulting in overestimation of the moisture content. On the other hand, certain oxidizing agents such as Cu^{2+}, Fe^{3+}, NO_2^-, Br_2, Cl_2 and quinines would oxidize I^- (one of the KF reaction products) to I_2, and thus the presence of these species in the matrix would lead to underestimation of the moisture content. In addition, I_2 can combine with I^- to produce the triiodide ion (I_3^-):

$$I_2 + I^- \longleftrightarrow I_3^- \qquad [2.4]$$

Although this ion will eventually react with water, it has been reported that reactions involving the triiodide ion are about 4-fold slower than those of iodine itself **(62)**, which may lead to underestimation of water content. To minimize the above-mentioned problems, it is strongly suggested to use fast-reacting KF reagents combined with optimum conditions in order to speed up the reaction so that less side reactions will have chance to occur **(63,64)**.

Another important modification of the KF method was that introduced to make it suitable for the determination of water in hydrophobic liquids such as edible oils and lubricants, because the KF reagents as originally formulated **(65)** were not miscible with

such samples. It was suggested to modify the polarity of the KF working medium by adding solvents such as chloroform **(66)**, xylene, hexanol, 1-propanol, 1-octanol **(67)**, propylene glycol and others. Partial solubilization of oil samples in KF reagents was found to be one of the main reasons for the commonly observed variation in results obtained by the KF method **(68)**.

Most of the progress that has been made in understanding and improving KF reactions has been adopted commercially. An important consideration in making commercial formulations is the toxicity of the reagents, and the pyridine employed in the original KF reagent has largely been replaced by the less noxious imidazole, which also serves to speed up the KF reaction, as discussed above. Different formulations of KF reagents are available under various brand names, the most common being HYDRANAL®, representing pyridine-free reagents, which are produced in different formulations to suit a variety of applications. On the other hand, this complicates the universal comparison of KF results as the results obtained depend on the formulation used.

KF reaction cell. In conjunction with the work done with reagent formulations to make the KF method faster and less susceptible to side reactions, substantial development has taken place in relation to the design of the reaction vessel. The conventional coulometric cell includes a potentiometric detector, a cathodic and an anodic compartment, and a diaphragm. The cathode is in electrolytic contact with the anode through the diaphragm, which plays a very important role in restricting the iodine that is being produced at the anode from reaching the cathode, where it can be reduced by reaction products such as thiosulfate, hydrogen sulfide and other products that are believed to be produced at the cathode **(69,70)**. Optimization of the dimensions of the cathode and anode and the possibility of removing the diaphragm have been extensively studied to make the design simpler and facilitate maintenance. As a result, many designs have been proposed with various cathode dimensions, with or without a diaphragm, and with the use of constant or pulsed currents **(71-79)**. The use of a diaphragm eliminates the possibility of iodine being reduced by products at the cathode and allows the use of electrolytes other than the KF reagent in the cathodic compartment of the cell. Clogging of the diaphragm, however, is a common problem and the cell requires long conditioning

times before startup (~2.5 h). On the other hand, diaphragm-free systems have shorter conditioning times and are easier to maintain, but they suffer from the formation of oxidizable reduction products at the cathode. This problem has been minimized considerably by implementing rapidly reacting reagents combined with optimizing the dimensions of the cathode and the current density. The latter were found to be critical factors in determining the analytical accuracy obtained with the coulometric cell **(72, 77-80)**. The type of current used in the coulometric cell, pulsed or constant, was found to be critical, especially for diaphragm-free cells, in reducing the rate of development of oxidizable products at the cathode and also to minimize the drift at the end point. In this regard, pulsed current was found to be the most suitable for diaphragm-free cells **(78-80)**. Investigation of such cells also indicated that the titration rate is a critical parameter in relation to analytical accuracy **(79)**, but the optimum varies from one system to another.

KF detectors. Many end point detectors have been used with the KF method. Determining which detector is best from the literature is not easy because comparisons are not straightforward as the end-point detection is influenced by a number of factors. These include the mode of titration (volumetric or coulometric), cell design, electrode response, the extent of background drift caused by moisture diffusion, side reactions and the use of different reagents and additives in the titration.

Early on, amperometric detectors were commonly in use, largely supplanted later **(81,82)** by controlled-current potentiometers (bipoteniometry) **(59, 83-86)**. In general, both these types of detectors use a constant current between two platinum electrodes while measuring the voltage needed to sustain the current. At the equivalence point of the titration, there is a sharp drop in the voltage required to sustain the current because excess I_2 is present and the current can be carried at a very low voltage in its presence. Although controlled-current potentiometers have better accuracy and shorter analysis times than amperometric detectors, their accuracy was found to be affected by both the electrode dimensions and the external current generated between the cathode and the anode. Recently, zero-current potentiometers (true potentiometry) have received substantial interest **(66,71,87,88)** because they offer better selectivity and do not suffer from the electric field interference generated in the coulometric cell. Although it is the detector of choice in modern KF coulometric cells, this type of detector must meet certain

requirements to perform well such as a suitable reference electrode system and a calibration procedure to establish the relationship between the redox potential and the concentration of excess iodine **(87)**.

Spectrophotometric end-point detection. Upon addition of the KF reagent to a sample, the original dark brown color due to the presence of iodine changes toward light yellow depending on the amount of water in the sample that has reacted with the KF reagent. This change in color implies that the development of a colorimetric method for the KF method would be possible; however, very little work has been done in this regard. The earliest attempt to employ colorimetric detection in KF titration involved measuring the absorbance of the sample at 525 nm **(89)**. This approach allowed detection of 100 ppm water with a precision of ±3 ppm. Subsequently, Dahms **(90)** patented a colorimetric method for determination of moisture using the KF reaction based on the injection of the sample into a fixed volume of KF reagent and colorimetric measurement of the change in optical density (OD) at 520 nm. The amount of water in the sample was determined from a calibration curve established by measuring the OD of the reagent mixed with defined amounts of water. Although the author used 520 nm as a working wavelength, he noted that the KF reagent absorbs over a wide spectral range (500-620 nm) and the sensitivity of the method depended on the wavelength used. This approach was adapted for use with a flow injection analysis (FIA) system **(91)** and improved to correct for factors affecting OD measurements such as dilution effects, the transmission characteristics of the cuvette, the refractive index of the sample, etc., through the use of a reference dye that has an absorption (600 nm) far from the working wavelength (set at 420 nm) **(92)**. A system based on this approach combined with specially designed ready-to-use sealed vials containing the KF reagent was developed **(93,94)**. Although this system appears simple to use, it has not been independently validated.

Flow injection analysis. Due to the many interferences affecting KF titrimetric methods, various approaches have been explored to carry out KF analysis. As indicated earlier, many authors suggested speeding up the KF reaction as a means to both overcome the problems of side reactions and increase accuracy by minimizing the problems inherent to coulometric analysis. These issues become even more important when one considers FIA as a means of automating KF moisture determinations. In 1980, Kågevall

et al. **(91)** developed an FIA system for determination of moisture in organic solvents using both potentiometric and spectrophotometric detectors. The method used a fast-reacting one-component KF reagent (formamide/pyridine buffer instead of pyridine alone) which was diluted with methanol as a carrier solvent. The system was capable of analyzing up to 120 samples/h and covered a range of 100-50,000 ppm moisture. The authors noted that the potentiometric detector gave a better SD (≤0.5%) than that obtained spectrophotometrically. The efficacy of the system in minimizing side reactions in iodine-consuming samples was tested using penicillin as the sample (95), with the results indicating a considerable decrease in side reactions. A similar, but improved FIA system was introduced by Escott and Taylor **(96)** to analyze gasoline-alcohol blends. A methanol-xylene mixture was used to dilute samples and make them miscible with the methanolic KF reagent. Quantification was based on direct potentiometric measurement and the system, which was equipped with an autosampler, was able to analyze up to 60 samples/h over a range of 1-1500 ppm moisture. The results tended to be higher than those obtained using a titrator, but the reproducibility was better. The variables affecting the performance of the KF method in the FIA mode using potentiometric or spectrophotometric detectors have been thoroughly investigated by Dantan *et al.* **(97)**. The authors suggested new FIA designs for both types of detectors to provide more flexibility in reagent concentrations as well as on-line analysis capability. The optimized methods permit rapid, precise and automated determinations of moisture in a wide range of samples over the range of 100-50,000 ppm with a reproducibility of better than 3% RSD. According to the authors, alteration of certain parameters, such as injection volume, detection wavelength and/or the concentration of KF reagent, could further extend the analytical range, with the presence of traces of water in the carrier solvent being the main factor determining the detection limits of these methods.

Current status of the KF method. As can be seen from the above discussion, most of the modifications of the KF method proposed to date have focused on minimizing the inherent problems associated with the KF reaction itself. These modifications have minimized the problems of side reactions and increased the diversity of samples that can be analyzed, but at the cost of complicating the method due to the introduction of different KF reagents and apparatus designs. The modifications that were introduced over

the years made the method suitable for the analysis of almost all types of samples; however, ultimately the accuracy of the method is dependent on selecting the right combinations of a long list of variables and operating parameters. The selection of variables is difficult because it requires not only knowing what is in the sample that is being analyzed but also understanding clearly the chemistry involved. In this regard, Margolis **(68,98)** noted that systematic biases were observed in interlaboratory collaborative studies and depended on both the type of apparatus and the nature of the solvent system employed in the measurement. He tested a number of hydrocarbon-based lubricating oils using different KF methods (volumetric and coulometric) and different analysis conditions (different solvent compositions and different instrumental parameters) and concluded that the measurements were very much dependent on the nature and concentration of the non-polar organic solvent and the miscibility of the oil with the KF reagent. The severity of the bias can be minimized by selecting the appropriate working parameters and reagent composition for the sample at hand, but these are not readily evident in many cases. Recently, Larsson and Cedergren **(99)** studied factors influencing the accuracy and precision of moisture determination in oil using a diaphragm-free KF coulometric cell using eight different types of commercial coulometric reagents and various modifications. The best results were obtained with a homemade coulometric cell with fully adjustable parameters and reagent formulations not commercially available. Although one of the authors (Cedergren) has spent more than 20 years working with the KF method, he declared that there is more research needed to better understand the function of the cathode reaction in the diaphragm-free coulometric cell when using different types of KF media. Aside from these issues, the coulometric cell has the disadvantage of requiring regular maintenance (weekly) and long conditioning times. Even with its many problems, the KF method remains the gold standard for moisture determination as no better alternative has been developed.

2.2.3. Infrared Methods

At first glance, FTIR spectroscopy would appear to be a viable technique for the measurement of moisture in oils, as water absorbs strongly in the IR portion of the spectrum (**Figure 2.7**), exhibiting intense and readily measurable bands in the regions of

3700-3000 and 1670-1630 cm^{-1} due to its O-H stretching and H-O-H bending vibrations, respectively (**100**).

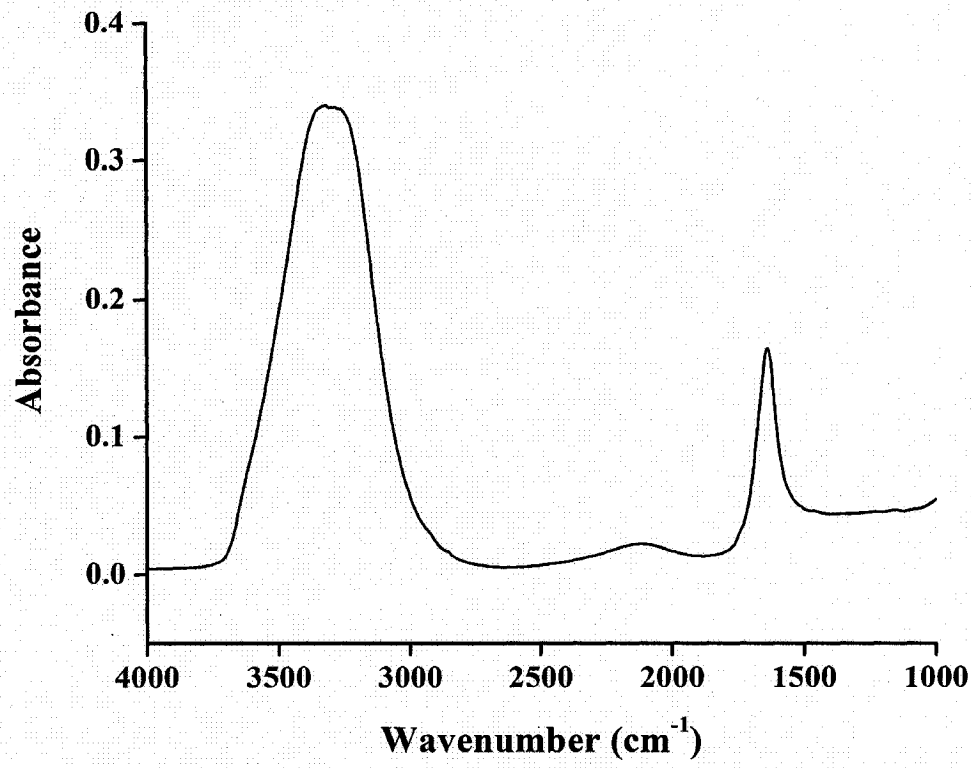
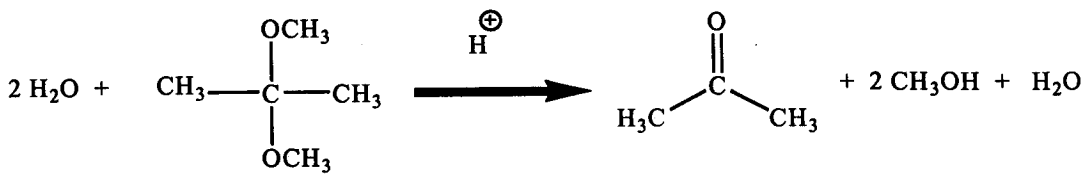


Figure 2.7. FTIR spectrum of pure water.

However, FTIR moisture analysis in oils is complicated by spectral interferences from other OH-containing constituents, such as FFA, mono- and diglycerides, alcohols, and hydroperoxides, which may be present in substantial amounts (in terms of their impact on moisture quantification both in crude oils and in refined oils that have undergone auto-oxidation to any significant extent). Quantification of moisture is further confounded by the hydrogen bonding interactions between water and these species as well as between water and carbonyl-containing secondary oxidation products (aldehydes and ketones), as such interactions affect the positions, band shapes, and intensities of the water absorptions. Chemometric techniques such as PLS can, in principle, be employed to compensate for these types of "matrix effects" but, in practice, their utility is limited by the difficulty of meeting the criterion that all possible sources of spectral variability must

be represented within the training set used to develop a calibration model. This limitation is well illustrated by previous work conducted by the McGill IR Group on the determination of hydroperoxides in edible oils based on their O-H stretching band at 3444 cm^{-1}, which is subject to effectively the same confounding effects as moisture analysis **(101,102)**. Recently, Che Man and Mirghani **(50)** published a PLS-based FTIR method for the determination of moisture in crude palm oil based on a set of calibration standards prepared by spiking oils with water to cover a range of 0-13% moisture content. The method is simple to implement, involving recording the spectra of neat oil samples in a 100-μm transmission cell and using the PLS algorithm to predict the moisture content. However, validation of this method by predicting an independent test set was not performed, and hence there is no evidence that unknowns would be modeled adequately by the limited number of spiked oil samples used to develop the PLS calibration. Indeed, the physical stability of the calibration standards, and hence the validity of the calibration model, is questionable since phase separation of oil standards having moisture contents as high as 13% would seem likely. Apart from these issues, this work does not serve as the basis for a global method, as the calibration model developed is specific for crude palm oil and does not take into account the complicating factors outlined above that must be considered in the development of a *generalized* FTIR method for the determination of moisture in edible oils.

A completely different approach to overcoming matrix effects was taken by van de Voort *et al.* **(103)** for the FTIR determination of moisture in new and used mineral-based lubricants, which are quite different matrices from edible oils but present similar challenges from an FTIR analytical perspective. The approach involves splitting the oil sample into two halves and treating one half with a dimethoxypropane (DMP)/dioxane mixture to convert water present in the oil into acetone via the reaction in Eq.2.5 prior to recording its FTIR spectrum.



2,2-dimethoxypropane

[2.5]

The other half of the sample is simply diluted with dioxane, and its spectrum is subtracted from that of the DMP-treated portion to generate a differential spectrum. The amount of acetone that is produced in the reaction is quantified by measuring its ν (C=O) absorption at 1740 cm^{-1} in the differential spectrum and is related to the amount of water present in the initial sample using a simple calibration equation. Owing to the high molar absorptivity of this acetone band, very good sensitivity (±50 ppm water) was obtained over the range 0-2000 ppm. Thus, the use of a reagent that reacts stoichiometrically with water to produce a strongly IR-absorbing product followed by the application of differential spectroscopy to isolate the spectral features associated with this conversion provided a generalized method for the determination of moisture in mineral-based lubricants, without the complexities inherent in chemometric modeling of the sample matrix. This approach, however, is not directly applicable to edible oils because the triacylglycerol ester linkages give rise to an intense ν (C=O) absorption that would completely mask the ν (C=O) band of acetone in a transmission-cell spectrum, except at extremely short path lengths. Moreover, the use of ATR, which inherently provides a short effective path length, to overcome this limitation is impractical owing to the difficulty of preventing the sample from picking up moisture from the environment or losing the acetone produced, nor would it provide sufficient sensitivity for the analysis of moisture at the low levels present in edible oils.

With regard to moisture analysis in edible oils by vibrational spectroscopic techniques other than FTIR spectroscopy, a few papers on NIR methods have appeared in the literature. The first paper, published in 1970 by Vornheder and Brabbs **(104)**, extended work published by other authors who determined water content in a variety of samples (organic solvents **(105)** and food materials **(106,107)**), but not edible oils. The method involves mixing oil samples with a polar solvent (DMSO) in order to extract water molecules from the oil. The intensity of the water absorption band in the NIR spectrum of the solvent is then measured in the range 2.1-1.8 μm (max absorption at 1.94 μm (5160 cm^{-1}), and the water content is determined using a simple Beer's law equation. Although the working range of the method depends on the sample:solvent ratio, the limit of detection of the method was 300 ppm. Recently, Cozzolino *et al.* **(46)** used PLS to

establish a method for moisture analysis in fish oil from the NIR spectra of neat oil samples. The calibration equation had R^2 of 0.8.

2.3. CONCLUSION

The standardized methods for the determination of both FFA and moisture in edible oils established in the middle of the twentieth century continue to be the basis for the determination of these crucial oil quality parameters. Many modifications of these methods or alternative techniques have been proposed, but it has been shown in the discussion above that none of these have fully addressed the need for rapid, accurate and simple instrumental methods that would be suited to automated process and quality control. FTIR spectroscopy has the potential to meet this requirement, but this promise has not yet been fulfilled in fairly extensive work to date on FFA analysis or in a single study on moisture analysis. Accordingly, the research described in the following chapters of this thesis was undertaken to overcome the limitations of the FTIR methods developed to date and allow the potential of FTIR analysis to be realized.

2.4. REFERENCES

1. O'Brien, R. D., *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing Company, Lancaster, PA, 1998.
2. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., Champaign, IL, 1989, AOCS Official Method Ca-5a-40.
3. Determination of the Free Fatty Acids in European Commission Regulation (EED) No. 2568/91, Official Journal of the European Communities, No. L 248 (5.9.91), p. 6.
4. Ayers, C. W. Estimation of the Higher Fatty Acids C_7-C_{18}. *Anal. Chim. Acta*, 15, 77-83 (1956).
5. Baker, D. A Colorimetric Method for Determining Free Fatty Acids in Vegetable Oils., *J. Am. Oil Chem. Soc.*, 41, 21-23 (1964).
6. Lowry, R. R. and Tinsley, I. J. Rapid Colorimetric Determination of Free Fatty Acids, *J. Am. Oil Chem. Soc.*, 53, 470-473 (1976).
7. Bains, G. S., Rao, S. V. and Bhatina, D. S. A Colorimetric Procedure for the Estimation of fat Acidity in Peanuts and Peanut Meals. *J. Am. Oil Chem. Soc.*, 41, 831-832 (1964).
8. Ekstrom, L-G. An Automated Method for Determination of Free Fatty Acids. *J. Am. Oil Chem. Soc.*, 58, 835-838 (1981).

9. Kwon, D. Y. and Rhee, J. S. A Simple and Rapid Colorimetric Method for Determination of Free Fatty Acids for Lipase Assay. *J. Am. Oil Chem. Soc.*, 63, 89-92 (1986).

10. Brenardez, M., Pastoriza, L., Sampedro, G., Herrera, J. J. and Cabo, M. L. Modified Methods for the Analysis of Free Fatty Acids in Fish. *J. Agric. Food Chem.*, 53, 1903-1906 (2003).

11. Canham, J. S. and Pacey, G. E. Automated Free Fatty Acids Determination Using Flow Injection Analysis Solvent Extractions. *J. Am. Oil Chem. Soc.*, 64, 1004-1007 (1987).

12. Puchades, R., Suescun, A. and Maquieira, A. Determination of Free Fatty Acids in Foods by Flow Injection. *J. Sci. Food Agric.*, 66, 473-478 (1994).

13. Zhi, Z-L, Ríos, A. and Valcárcel, M. An automated Flow Reversal Injection/Liquid-Liquid Extraction Approach to the Direct Determination of Total Free Fatty Acids in Olive Oils. *Anal. Chim. Acta*, 318, 187-194 (1996).

14. Linares, P., Luque de castro, M. D. and Valcárcel, M. Direct Automatic Determination of Free Acidity in Oils Flow-Injection Analysis. *Anal. Chim. Acta*, 255, 431-436 (1989).

15. Nouros, P. G., Constantinos, A. G. and Polissiou, M. G. Automated Flow Injection Spectrophotometric Non-Aqueous Titrimetric Determination of the Free Fatty Acid Content of Olive Oil. *Anal. Chim. Acta*, 351, 291-297 (1997).

16. Mariotti, E. and Mascini, M. Determination of Extra Virgin Olive Oil Acidity by FIA-Titration. *Food Chem.*, 73, 235-238 (2001).

17. Walde, P. A Colorimetric Determination of Fatty Acids as a New Assay of Lipase in Reverse Micelles. *J. Am. Oil Chem. Soc.*, 67, 110-115 (1990).

18. Walde, P. and Nastruzzi, C. Application of a New, Simple and Economic Colorimetric Method for the Determination of Non-Esterified Fatty Acids in Vegetable Oils. *Food Chem.*, 39, 249-256 (1991).

19. Takamura, K. and Hayakawa, Y. Determination of Traces of Free Acid by the Polarographic Reduction of Quinones. *Microchem. J.*, 17, 546-550. (1972).

20. Kusu, F., Fuse, T. and Takamura, K. Voltammetric Determination of Acid Values of Fats and Oils. *J. AOAC Int.*, 77, 1686-1689 (1994).

21. Fuse, T., Kusu, F. and Takamura, K. Voltammetric Determination of Free Fatty Acid Content in Fats and Oils. *Bunseki Kagaku*, 44, 29-33 (1995).

22. Fuse, T., Kusu, F. and Takamura, K. Determination of Higher Fatty acids in Oils by high-Performance liquid Chromatography with Electrochemical detection. *J. Chromatogr.*, 764, 177-182 (1997).

23. Fuse, T., Kusu, F. and Takamura, K. Determination of Acid Values in Fats and Oils by Flow Injection Analysis with Electrochemical Detection. *J. Pharm. Biomed. Anal.*, 15, 1515-1519 (1997).

24. Takamura, K., Fuse, T., Arai, K. and Kusu, F. A Review of a New Voltammetric Method for Determining Acids. *J. Electroanal. Chem.*, 468, 53-63 (1999).

25. Kotani, A., Kusu, F. and Takamura, K. New Elecrochemical Detection Method in High-performance Liquid Chromatography for Determining Free Fatty Acids. *Anal. Chim. Acta*, 465, 199-206 (2002).

26. Lapshina, T. M., Tur'yan, Y. I. and Danilchuk, S. I. pH-Metric Determination of Acid Numbers for Oils. *J. Anal. Chem. USSR*, 46, 1150-1158 (1991).

27. Tur'yan, Ya. I., Berezin, O. Yu., Kuselman, I. and Shenhar, A. pH-Metric Determination of Acid Values in Vegetable Oils without Titration. *J. Am. Oil Chem. Soc.*, 73, 295-301 (1996).

28. Tur'yan, Ya. I., Strochkova, E., Berezin, O. Yu., Kuselman, I. and Shenhar, A. pH-Metric Determination of Acid Numbers in Petroleum without Titration. *Talanta*, 47, 53-58 (1998).

29. Kuselman, I,.Tur'yan, Ya. I., Burenko, I. and Anisimov, B. pH-Metric Determination of Acid Values in Oilseeds without Titration. *Talanta*, 49, 629-637 (1999).

30. Strochkova, E. Tur'yan, Ya. I. and Kuselman, I. pH-Metric Determination for Acid Number in Hydraulic Oils without Titration. *Talanta*, 50, 1135-1139 (1999).

31. Lingane, J. J. Automatic Potentiometric Titrations. *Anal. Chem.*, 20, 285-292 (1948).

32. Muller, R. H. Electronic Trigger Circuit for Automatic Potentiometric and Photometric Titrations. *Anal. Chem.*, 20, 795-797 (1948).

33. Shaffer, P. A., Briglio, A. and Brockman, A. J. Instrument for Automatic Continuous Titration. *Anal. Chem.*, 20, 1008-1014 (1948).

34. Delahay, P. Electrical Differential Method of Measurement in Electrotitrations: Application to Potentiometric Titrations. *Anal. Chem.*, 20, 1212-1215 (1948).

35. Furman, N. H. Potentiometric Titrations. *Anal. Chem.*, 22, 33-41 (1950).

36. http://www.mt.com/mt/resourcedetail/appEdStyle.jsp?m=t&key=I2Mjg4NjM1Mj

37. Vaughan, G. A. and Swithenbank, J. J., Acetone as Solvent in the Enthalpimetric Titration of Acidic Substances. *Analyst*, 90, 594-599 (1965).

38. Thomas, K. S. Analysis of FFA in Edible Oils by Catalyzed End-Point Thermometric Titrimetry (CETT). *J. Am. Oil Chem. Soc.*, 80, 21-24 (2003).

39. Lanser, A. C., List, G. R., Holloway, R. K. and Mounts, T. L. FTIR Estimation of Free Fatty Acid Content in Crude Oils Extracted from Damaged Soybeans, *J. Am. Oil Chem. Soc.*, 68, 448-449 (1991).

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

41. Man, Y. B., Moh, M. H. and van de Voort, F. R. Determination of Free Fatty Acids in Crude Palm Oil and Refined-Bleached-Deodorized Palm Olein Using Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 76, 485-490 (1999).

42. Bertran, E., Blanco, M., Coello, J., Iturriaga, H., Maspoch, S. and Montoliu, I. Determination of Olive Oil Free Fatty Acid by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 76, 611-616 (1999).

43. Verleyen, T., Verhe, R., Cano, A., Huyghebaert, A. and De Greyt, W. Influence of Triacylglycerol Characteristics on the Determination of Free Fatty Acids in Vegetable Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 78, 981-984 (2001).

44. Cañada, M. J. A., Medina, A. R. and Lendl, B. Determination of Free Fatty Acids in Edible Oils by Continuous-Flow Analysis with FT-IR Spectroscopic Detection, *Appl. Spectrosc.*, 55, 356-360 (2001).

45. Zhang, H-Z and Lee, T-C. Rapid Near-Infrared Spectroscopic Method for the Determination of Fatty Acid in Fish and Its Application in Fish Quality Assessment. *J. Agric. Food Chem.*, 45, 3515-3521 (1997).

46. Cozzolino, D., Murray, I., Chree, A. and Scaife, J. R. Multivariate Determination of Free Fatty Acids and Moisture in Fish Oils by Partial Least-Ssquares Regression and Near-Infrared Spectroscopy. *LWT Food Sci. Technol.*, 38, 821-828 (2005).

47. Man, Y. B. and Moh, M. H. Determination of Free Fatty Acids in Palm Oil by Near-Infrared Reflectance Spectroscopy. *J. Am. Oil Chem. Soc.*, 75, 557-562 (1998).

48. Muik, B., Lendl, B., M-D, A. and Cañada, M. J. Direct, Reagent-Free Determination of Free Fatty Acid Content in Olive Oil and Olives by Fourier Transform Raman Spectrometry. *Anal. Chim. Acta*, 487, 211-220 (2003).

49. Mailer, R. J. Rapid Evaluation of Olive Oil Quality by NIR Reflectance Spectroscopy. *J. Am. Oil Chem. Soc.*, 81, 823-827 (2004).

50. Man, Y. B. C and Mirghani, M. E. S., Rapid Method for Determining Moisture Content in Crude Palm Oil by Fourier Transform Infrared Spectroscopy. *J. Am. Oil Chem. Soc.*, 77, 631-637 (2000).

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

52. Scholz, E. Karl Fischer Titrations of Aldehydes and Ketones. *Anal. Chem.*, 57, 2965-2971 (1985).

53. Fischer, F. and Schiene, R. Dead-Stop Determination of Traces of Water in Aliphatic Ketones with Karl Fischer Solution. *Zeitschrift fuer Chemie*, 4 (2), 69-70 (1964).

54. Klimova, V. A., Sherman, F. B. and L'vov, A. M. Micro-Determination of Water by Fischer Reagent Prepared with N,N'-Dimethylformamide Instead of Methanol. *Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya,* 12, 2599-2602 (1967).

55. Mitsubishi Chemical Industries, German Patent Application DE 30 40 474 (1980).

56. Hydranal Composite 5K, leaflet from Riedel de Haen AG, D-3016 seeize.

57. Nordin-Andersson, I. and Cedergren, A. Coulometric Determination of Trace Water in Active Carbonyl Compounds Using Modified Karl Fischer Reagents. *Anal. Chem.*, 59, 749-753 (1987).

58. Bizot, J. Automatic Method for Coulometric Determination of Minute Quantities of Water. *Bull. Soc. Chim. France,* 151- 157 (1967).

59. Cedergren, A. Reaction Rates Between Water and some Modified Rapidly-Reacting Karl Fischer Reagents. *Talanta,* 25, 229-232 (1978).

60. Scholz, E. Karl-Fischer reagents without pyridine. 3. Accuracy of the Determination of Water. *Fresenius Z. Anal. Chem.*, 306, 394-396 (1981).

61. Orädd, C. and Cedergren, A. Reaction Rates of Water in Imidazole-Buffered Methanolic Karl Fischer Reagents. *Anal. Chem.*, 66, 2603-2607 (1994).

62. Verhoef, J. C. and Bahrendrecht, E. Mechanism and Reaction Rate of the Karl-Fischer Titration Reaction. Part I. Potentiometric Measurements *J. Electroanal. Chem.*, 71, 305-315 (1976).

63. Cedergren, A. and Orädd, C. Coulometric Study of the Influence of Aldehydes and Ketones on the Karl Fischer Titration of Water Using Standard Pyridine /Methanol Reagents. *Anal. Chem.*, 66, 2010-2016 (1994).

64. Orädd, C. and Cedergren, A. Coulometric Study of the Recovery Rates for Karl Fischer Titration of Water in Aldehydes and Ketones Using Rapidly Reacting Methanolic and 2-Methoxyethanolic Reagents. *Anal. Chem.*, 67, 999-1004 (1995).

65. Fischer, K. A New Method for the Analytical Determination of the Water Content of Liquids and Solids. *Angew. Chem.*, 48, 394-396 (1935).

66. Cedergren, A. Coulometric Study of Reaction Rates of Water in Pyridine and Imidazole-Buffered Methanolic Karl Fischer Reagents Containing Chloroform. *Anal. Chem.*, 69, 3682-5687 (1996).

67. Isengard, H-D and Kerwin, K. Proposal of a New Reference Method for Determining Water Content in Butter Oil. *Food Chem.*, 82, 117-119 (2003).

68. Margolis, S. Amperometric Measurement of Moisture in Transformer Oil using Karl Fischer Reagents. *Anal. Chem.*, 67, 4239-4246 (1995).

69. Katoh, H. Fujimoto, Y. and Kuwata, Y. A Study of the Coulometric Cathode Reaction in the Karl Fischer Reaction. *Anal. Sci.* 7, 299-302 (1991).

70. Scholz, E. Karl Fischer Coulometry – the Cathode Reaction. *Fresenius J. Anal. Chem.* 348, 269-271 (1994).

71. Cedergren, A. and Jonsson, S. Diaphragm-Free Cell for Trace Determination of Water Based on the Karl Fischer Reaction using Continuous Coulometric Titration. *Anal. Chem.* 69, 3100-3108 (1997).

72. Cedergren, A. and Jonsson, S. Progress in Karl Fischer Coulometry using Diaphragm-Free Cells. *Anal. Chem.* 73, 5611-5615 (2001).

73. Dahms, H. High Current Coulometric KF Titrator. US Patent 5300207 (1994).

74. Nichugovskii, G. F. Coulometric Cell of Simplified Design for Titration of Water in a Fischer's Reagent Medium *Zh. Anal. Khim.* 32, 2124-2126 (1977).

75. Katoh, H. Fujimoto, Y. and Kakuda, M. Effects of the Cathode Current Density in the Karl Fischer Coulometric Titration Method. *Anal. Sci.* 8, 575-577 (1992).

76. Nichugovskii, G. F. and Yakolev, P. P. Coulometric Determination of Water with Fischer's Reagent in a One-Chamber Cell. *Zh. Anal. Khim.*, 33, 684-687 (1978).

77. Nordmark, U. and Cedergren, A. Conditions for Accurate Karl Fischer Coulometry using Diaphragm-Free Cells. *Anal. Chem.* 72, 172-179 (2000).

78. Nordmark, U., Rosvall, M. and Cedergren, A. Optimum Conditions for Pulse Generation in Daphram-Free Karl Fischer Coulometry. *Fresenius J. Anal. Chem.*, 368, 456-460 (2000).

79. Nordmark, U. and Cedergren, A. Progress in Pulsed-Current Karl Fischer Coulometry using Diaphragm-Free Cells. *Fresenius J. Anal. Chem.*, 367, 519-524 (2000).

80. Borsten, H. and van Lamoen, F. L. J. Potentiometric Method for Karl Fischer Titration. *Anal. Chem.*, 27, 1639-1640 (1955).

81. Brown, J. F. and Volume, W. F. An Automatic Fischer Titration Unit for Laboratory Use. *Analyst*, 81, 308-315 (1956).

82. Cedergren, A. Coulometric Trace Determination of Water by using Karl Fischer Regent and Potentiometric End-Point Detection. *Talanta*, 21, 367-375 (1974).

83. Lindbeck, M. R. and Freund, H. Microdetermination of Water, using Rapid Controlled Potential Coulometry in a Karl Fischer System. *Anal. Chem.*, 37, 1647-1650 (1965).

84. Cedergren, A. Comparison between Amperometric and True Potentiometric End-Point Detection in the Determination of Water by the Karl Fischer Method. *Talanta*, 21, 553-563 (1972).

85. Harris, D. C. *Quantitative Chemical Analysis*, 5th edn., W. H. Freeman and Company, New York, 1999, pp. 498-499

86. Cedergren, A. Comparison between Bipotentiometric and True Potentiometric End-Point Detection using Rapidly Reacting Karl Fischer Reagents. *Anal. Chem.*, 68, 3679-3681 (1996).

87. Rosvall, M., Landmarl, L. and Cedergren, A. Computer-Controlled, Coulometric Karl Fischer Systems for Continuous Titration of Water Based on Zero Current Potentiometry. *Anal. Chem.*, 70, 5332-5338 (1998).

88. Bakker, E. and Pretsch, E. Potentiometry at Trace Levels. *Trends Anal. Chem.*, 20, 11-19 (2001).

89. Connors, K. A. and Higuchi, T. Spectrophotometric Detection of the End Point in the Karl Fischer Titration of Water. *Chemist-Analyst*, 48, 91-92 (1959).

90. Dahms, H. Method and apparatus for colorimetric analysis. US Patent 4005983 (1977).

91. Kågevall, I., Åström, O. and Cedergren, A. Determination of Water by Flow-Injection Analysis with Karl Fischer Reagent. *Anal. Chim. Acta*, 114, 199-208 (1980).

92. Dahms, Harald. Method for colorimetric determination of water using Karl Fischer reagents and optical density measurements at two different wavelengths. US Patent 4786602 (1988).

93. Dahms, Harald. Improved Colorimetric Karl Fischer Water Determination. PCT Int. Appl., WO 9221969 (1992).

94. Dahms, Harald. Sealed Vials Containing Improved Karl Fischer Solutions and Process for Water Determination using these Vials. US Patent 5179024 (1993).

95. Kågevall, I., Åström, O. and Cedergren, A. Minimization of Interference Effects from Iodine-Consuming Samples in the Determination of Water with the Karl Fischer Reagent in a Flow-Injection System. *Anal. Chim. Acta*, 132, 215-218 (1981).

96. Escott, R. E. and Taylor, A. F. Determination of Water by Flow Injection Analysis using Karl Fischer Reagent with Electrochemical Detection. *Analyst*, 110, 847- 849 (1985).

97. Dantan, N., Kröning, S., Frenzel, W. and Küppers, S. Comparison of Spectrophotometric and Potentiometric Detection for the Determination of Water using Karl Fischer Method under Flow Injection Analysis Conditions. *Anal. Chim. Acta*, 420, 133-142 (2000).

98. Margolis, S. Effect of Hydrocarbon Composition on the Measurement of Water in Oils by Coulometric and Volumetric Karl Fischer Methods. *Anal. Chem.*, 70, 4264-4270 (1998).

99. Larsson, W. and Cedergren, A. Coulometric Karl Fischer Titration of Trace Water in Diaphragm-Free Cells. *Talanta*, 65, 1349-1354 (2005).

100. Smith, B. *Infrared Spectral Interpretation: A Systematic Approach*, CRC Press, Boca Raton, FL, 1999, Chap. 1, p. 22.

101. van de Voort, F. R., Ismail, A. A., Sedman, J., Dubois, J. and Nicodemo, T. The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy. *J. Am. Oil Chem. Soc.*, 71, 815-826 (1993).

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

103. van de Voort, F. R., Sedman, J., Yaylayan, V., Saint Laurent, C. and Mucciardi, C. Quantitative Determination of Moisture in Lubricants by Fourier Transform Infrared Spectroscopy. *Appl. Spectrosc.*, 58, 193-198 (2004).

104. Vornheder, P. F. and Brabbs, W. J. Moisture Determination by Near-Infrared Spectrometry. *Anal. Chem.*, 42, 1454-1456 (1970).

105. Keyworth, D. A. Determination of Water by Near-Infrared Spectrophotometry. *Talanta*, 8, 461-469 (1961).

106. Rader, B. R. Determination of Moisture in Dried Vegetables and Spices. *J. Assoc. Offic. Anal. Chem.*, 49, 726-730 (1966).

107. Gold, H. J. General Application of Near-infrared Moisture Analysis to Fruit and Vegetable Materials. *Food Technol.*, 18(4), 184-185 (1964).

CHAPTER 3

NEW FTIR METHOD FOR THE DETERMINATION OF FREE FATTY ACID IN OILS

3.1. ABSTRACT

A rapid, practical and accurate FTIR method for the determination of free fatty acids (FFA) in edible oils has been developed. Analogous to the AOCS titration procedure, FTIR FFA determination is effected by an acid/base reaction but directly measures the product formed rather than utilizing an endpoint based on an electrode potential or color change. A suspension of a weak base, potassium phthalimide (K-phthal) in 1-PrOH, is used to convert FFA present in oils to their carboxylate salts without causing oil saponification, and differential spectroscopy is used to circumvent matrix effects. Samples are first diluted with 1-PrOH, then split, with one half treated with the K-phthal reagent and the other half with 1-PrOH (blank reagent), their spectra collected, and a differential spectrum obtained to ratio out the invariant spectral contributions from the oil sample. Quantitation of the percentage of FFA in the oil, expressed as % oleic acid, based on measurement of the peak height of the ν (COO^-) absorption of the FFA salt formed yielded a calibration with a standard error of <0.020 % FFA over the range of 0-4%. The method was validated by standard addition and the analysis of Smalley check samples, the results indicating that the analytical performance of the FTIR procedure is as good as or better than that of the standard titrimetric procedure. As structured, the FTIR procedure is a primary method, as calibration is not dependent on reference values provided by another method, and has performance criteria which could lead to its consideration as an instrumental AOCS procedure for FFA determination. The FTIR portion of the analysis is automatable, and a system capable of analyzing ~60 samples/h has been developed that could be of benefit to laboratories that carry out a large number of FFA analyses per day.

3.2. INTRODUCTION

FTIR spectroscopy is playing an increasingly important role in the analysis of edible oils by providing simpler and more rapid techniques for determining common oil quality parameters **(1)**. FFA are common triacylglycerol (TAG) hydrolysis products in

crude oils and are formed to some extent in refined oils as a result of oxidation or TAG degradation during frying, impairing oil quality and functionality. Chemically, FFAs are less stable than TAG and therefore more likely to oxidize and cause rancidity **(2)**. The standard method commonly used for FFA analysis is based on the titration of an oil dissolved in alcohol with a strong base to a phenolphthalein endpoint **(3,4)**. Although simple, titrimetric methods are tedious, consume substantial amounts of solvent, and can be problematic when dark crude oils are analyzed. The first application of FTIR spectroscopy for FFA analysis was reported by Lanser *et al.* **(5)** in 1991. In this method, which was developed for crude soybean oil, the FFA content was estimated from the FFA ν (C=O) band at 1710 cm^{-1}. Owing to the overlap of this band with the very strong TAG ester carbonyl absorption at 1746 cm^{-1}, spectral deconvolution over the 2000-1600 cm^{-1} range was used to mathematically enhance spectral resolution. A calibration was derived by spiking oleic acid into soybean oil at levels of 0.1-5% and yielded predictions of the FFA content of soybean oils that matched the values obtained using the AOCS titrimetric method to within ±0.5 percentage points. However, because the FTIR spectra were acquired by simply placing each sample between two KBr windows, without the use of an internal standard, the accuracy of this method was limited by the resulting variability in pathlength. In 1993, Ismail *et al.* **(6)** investigated two different FTIR approaches to the quantitative determination of FFA in edible oils. The first was based on measuring the carboxylic acid ν (C=O) band at 1711 cm^{-1} in spectra acquired from oil samples applied in their neat form onto an attenuated total reflectance (ATR) crystal. Both calibration and sample spectra were ratioed against the spectrum of an FFA-free oil of the same type as the oil being analyzed to reduce matrix effects. The second approach was an indirect method based on the use of KOH/methanol to extract the FFA present in the oil and convert them to their salts, followed by measurement of the ν (COO^-) absorption band at 1570 cm^{-1} in the spectrum of the methanol phase. This indirect method enhanced the sensitivity of the analysis by concentrating the FFA in a small volume of methanol and by utilizing an absorption band that is in a region free from major oil spectral interferences. However, it had the disadvantage of requiring an additional procedural step, and some saponification of oils by the KOH/methanol reagent added was noted, resulting in overestimation of the FFA content of the original sample. Verleyen *et al.* **(7)**

devised a more rigorous version of the method of Lanser *et al.* using peak height measurements at 1711 cm^{-1} to develop workable calibrations for a variety of oils but ultimately concluded that the calibrations were strongly oil dependent. Two other publications have dealt with FFA analysis by FTIR spectroscopy, specifically in relation to palm and olive oil, respectively **(8,9)**, both using partial least-squares regression (PLS) to develop relationships between spectral changes and results obtained by standard methods. The most sophisticated methodology is that of Cañada *et al.* **(10)**, who developed an automated FTIR-based continuous-flow analysis system capable of analyzing ~40 samples per hour using the indirect approach described by Ismail *et al.* **(6)**. However, even within the ~90 s required for analysis in this automated system, some saponification of the oil can occur. In general, the main drawback associated with the direct FTIR methods is their oil dependency, while the indirect FTIR methods are limited by the possibility of errors due to saponification caused by the KOH/methanol reagent. Hence, a simple, reliable, and robust FTIR method for FFA analysis is still lacking.

The McGill IR Group recently developed an FTIR-based instrumental method to replace the American Society for Testing and Materials (ASTM) titrimetric procedures for the determination of acid number (AN) in mineral and ester-based lubricating oils **(11)**. These ASTM methods are similar to those traditionally employed for the determination of FFA content in edible oils except that they measure not only carboxylic acids but also a variety of other acidic constituents, organic or inorganic, that accumulate in lubricating oils either as a result of oxidation or as combustion by-products **(12)**. Because the FTIR AN method was specifically designed to meet the requirements of lubricant analysis, it is not directly applicable to edible oils owing to their different spectral characteristics. However, elements of this methodology have been adapted to develop a new method for FFA determination that overcomes the limitations of both the direct and indirect FTIR methods previously developed for FFA analysis. This paper describes the principles of this method as well as their practical implementation and provides an evaluation of its performance by standard addition as well as by employing AOCS Smalley check samples.

3.3. EXPERIMENTAL PROCEDURE

Reagents and Standard Methods. All reagents used were of analytical grade. Potassium phthalimide (K-phthal, 99+%) and hexanoic acid (99%) were obtained from Aldrich (St. Louis, MO); 1-propanol (1-PrOH) and *iso*-propanol were purchased from Fisher Scientific Ltd. (Nepean, ON, Canada). All edible oils were obtained locally and samples were analyzed for FFA using AOCS method Ca 5a-40 **(3)**. Mineral oil (C-171 polyalphaolefin) was obtained from Thermal-Lube (Montreal, QC, Canada) and used for reagent blank determinations. A series of five oils pre-analyzed for FFA content were obtained from the AOCS Smalley Check Sample program.

Instrumentation. The instrument used for this study was a Bomem WorkIR spectrometer (Bomem, Quebec, QC, Canada) equipped with a DTGS detector and purged with dry air from a Balston dryer (Balston, Lexington, MA). Samples were analyzed by aspirating them into a 500 μm CaF_2 transmission flow cell mounted on a sample shuttle (Dwight Analytical, Toronto, ON, Canada). The spectrometer was controlled by an IBM-compatible Pentium 150-MHz PC running under proprietary Windows-based UMPIRE® (Universal Method Platform for InfraRed Evaluation) software (Thermal-Lube, Pointe-Claire, QC, Canada). All spectra were collected by co-adding 16 scans at a resolution of 8 cm^{-1} and a gain of 1.0.

Preparation of Calibration Standards. A series of eight standards covering the range 0-4% FFA (expressed as percentage of oleic acid) were prepared by gravimetric addition of hexanoic acid to a refined and deodorized soybean oil. The calibration curve was obtained by linear regression of % oleic acid (%FFA) against the peak heights in the FTIR spectra recorded for the standards by following the sample preparation and analytical protocols described below.

Sample Preparation for FTIR Analysis. Six grams of the oil sample was mixed with 3 ml of 1-PrOH in a 20-mL vial. Three-milliliter aliquots of the diluted oil were placed in two centrifuge tubes, labeled BR (blank reagent) and RR (reactive reagent), to which were added 7 mL of 1-PrOH and 7 mL of K-phthal/1-PrOH (20 g/L), respectively. All tubes were capped, shaken on a vortex mixer, and then centrifuged for a minimum of 5 min at 6000 rpm in a clinical centrifuge. It should be noted that K-phthal is virtually

insoluble in 1-PrOH and was dispensed via bottle re-pipette as a fine dispersion which was maintained by continuous and vigorous agitation on a magnetic stirrer.

Analytical Protocol. The transmission flow cell was loaded with ~2 mL of the BR sample and its single-beam spectrum was recorded. After the cell was flushed with *iso*-propanol, ~2 mL of the RR sample was loaded into the cell and its single-beam spectrum was recorded and ratioed against that of its corresponding BR sample to produce a differential spectrum. For quantitation of FFA, the peak height of the carboxylate ν (COO^-) band at 1570 cm^{-1} in the differential spectrum was measured relative to a baseline point at 2150 cm^{-1}. A schematic diagram of the sample preparation procedure and analysis is illustrated in **Figure 3.1**.

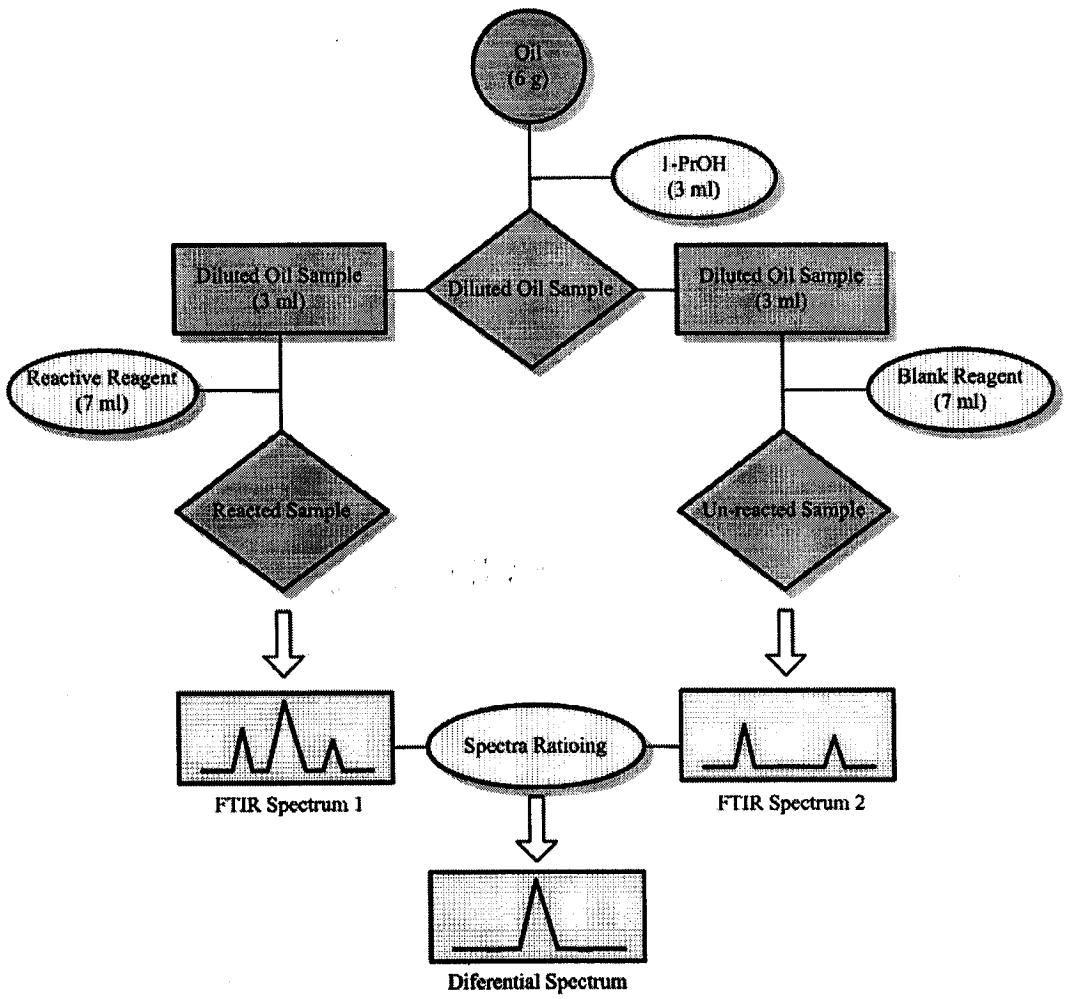


Figure 3.1. Schematic diagram illustrating the sample preparation procedure and the steps in the analytical protocol leading to the differential spectrum.

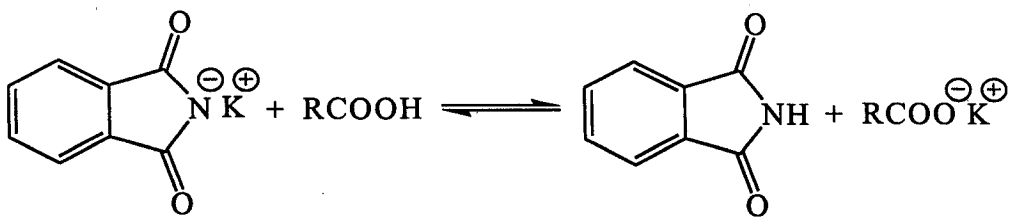
Validation. The FTIR method was validated by standard addition of FFA to soybean and corn oil and by analyzing AOCS Smalley check samples, which included five types of oils (crude coconut, crude corn, crude safflower, cottonseed, and marine oils). For the standard addition experiments, an FFA mixture was prepared by saponifying olive oil with 50% w/v KOH followed by titration with 6M HCl to regenerate the FFA **(13)**, extraction into n-hexane, removal of the solvent using a rotary evaporator, and titration of the residue to determine %FFA (expressed as % oleic acid). Soybean and corn oils were then spiked (w/w) with six levels of this FFA mixture. These samples and the Smalley check samples were analyzed for their FFA content by the FTIR method as well as by the AOCS titrimetric method. Reproducibility was evaluated as the standard deviation around the mean of triplicate analyses (SD_r). For the standard addition experiments, accuracy was assessed in terms of mean difference (MD_a) and standard deviation of the differences (SDD_a) with respect to the gravimetrically spiked amounts of FFA. In the case of the Smalley check samples, the FTIR results were compared to the results of certified laboratories that had analyzed the samples by the AOCS titrimetric procedure, using the statistical data (mean, min and max) provided with the samples.

3.4. RESULTS AND DISCUSSION

3.4.1. Analytical Concepts

As noted, FTIR methods developed to date for FFA analysis have been either matrix dependent or prone to saponification errors. In FTIR methodology developed for AN analysis in lubricants, the weak base K-phthal, a dicarbonyl compound, served as a *signal-transducing reagent by* reacting with all organic and inorganic acids present to form a single product, phthalimide, allowing AN to be determined from the intensity of phthalimide's strong ν (C=O) band at 1729 cm^{-1}, with differential spectroscopy then being used to eliminate the oil matrix effects **(12)**, This method is not directly applicable to edible oils, because the 1729 cm^{-1} phthalimide band is masked by overwhelming absorptions of the ester linkages of the TAG, whereas the second phthalimide ν (C=O) band at 1773 cm^{-1} is too weak to measure FFA concentrations of <1%. However, for the analysis of FFA, signal transduction is not required per se, only the stoichiometric conversion of FFA to their salts, which can then be quantitated directly by measurement

of their ν (COO^-) absorption. Moreover, the base used should be too weak to hydrolyze TAG, unlike the KOH employed in previous work **(6,10)**. K-phthal ($pK_a = 9.9$) was found to meet this criterion, with the additional advantage that 1-PrOH could be used to deliver this reagent into oils, thereby eliminating phase separation, with the FFA salts remaining soluble in the 1-PrOH/oil mixture. The stoichiometric reaction of K-phthal with FFA is presented below:



As in the lubricant AN methodology, the problem of matrix effects, which may arise in analyzing different oil types or result from the presence of various minor constituents in the samples analyzed, is addressed by utilizing differential spectroscopy. This approach involves splitting the sample into two parts; one part is then treated with K-phthal in 1-PrOH (designated RR) while the other portion is treated only with an equivalent amount of 1-PrOH (designated BR) and serves as a reference for the reacted sample. Since the spectral features of the oil are invariant in the spectra of the BR- and RR-treated portions, they are cancelled out in the differential spectrum and only the spectral changes associated with the acid/base reaction are left for evaluation (**Figure 3.2**).

3.4.2. Calibration and Stability of the Reaction

Figure 3.2 illustrates typical differential spectra obtained when calibration standards (soybean oil spiked with hexanoic acid over a range of 0-4% FFA) were analyzed using the protocol described above. As the amount of hexanoic acid increases in each subsequent standard, the ν (COO^-) signal at 1570 cm^{-1} and that of the phthalimide ν (C=O) band at 1773 cm^{-1} rise concurrently. The loss of the ν (C=O) band of hexanoic acid, which would manifest itself as a strong negative band around 1710 cm^{-1}, is largely

lost in the noise (1770-1700 cm^{-1}) resulting from the subtraction of the off-scale bands of the ester linkage of the oil.

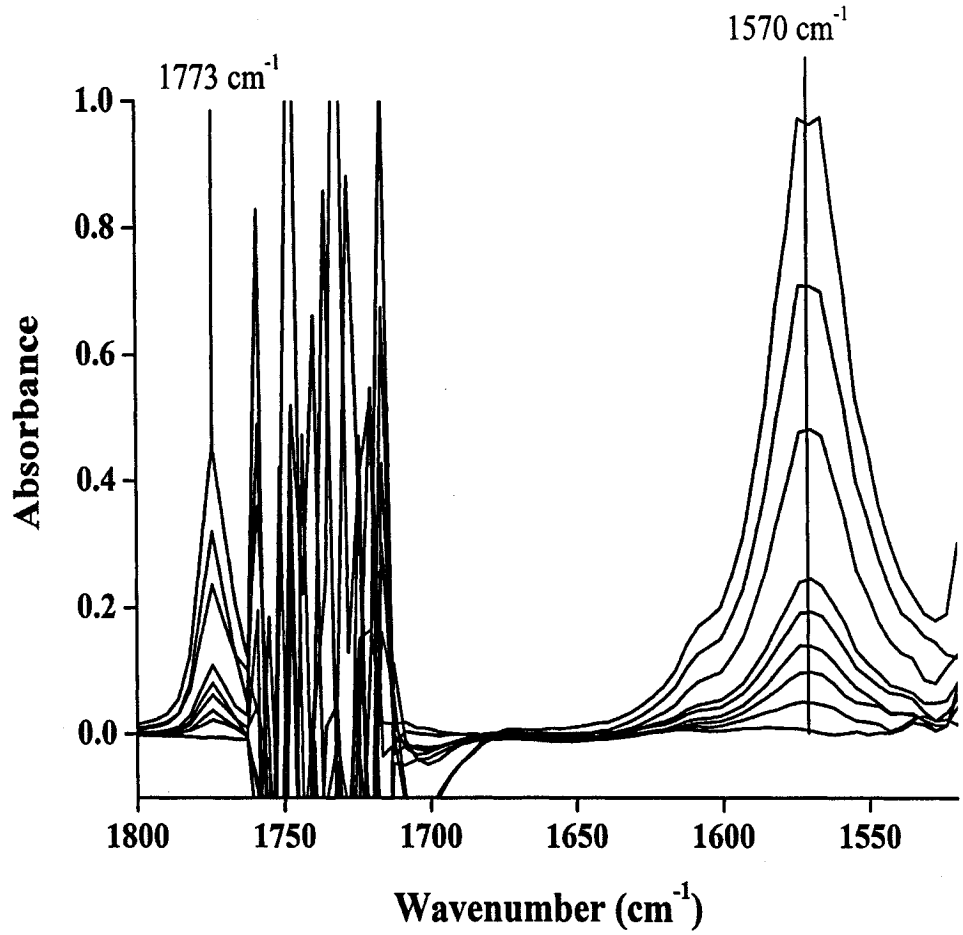


Figure 3.2. Potassium phthalimide as a reagent to carry out the acid/base reaction. Spectra were recorded in a 500 μm cell at 8 cm^{-1} resolution. The bands at 1773 and 1570 cm^{-1} are due to the phthalimide and hexanoate, respectively, formed in the reaction. The region between 1760 and 1710 cm^{-1} is obscured by noise in the differential spectrum because of the intense oil absorption in this region.

A mean plot with SD bars for three sets of hexanoic acid calibration standards obtained by measuring the ν (COO^-) band at 1570 cm^{-1} referenced to 2150 cm^{-1} *vs.* FFA concentration expressed as % oleic acid is presented in **Figure 3.3**. The overall linear regression equation obtained for the composite calibration was:

$$\% \text{ FFA}_{(\text{Oleic})} = 4.281 * A_{(1570/2150)} - 0.0436$$

$$R^2 = 0.999 \qquad \text{SD} = 0.020 \qquad [3.1]$$

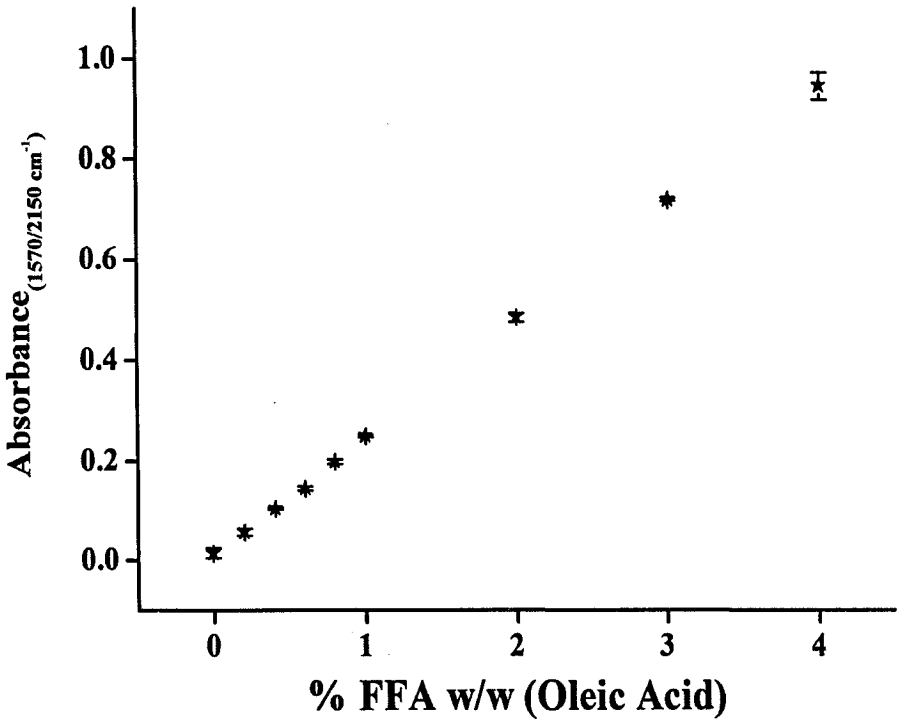


Figure 3.3. Composite calibration curve obtained from the differential spectra of three independent sets of hexanoic acid-spiked soybean oil standards of the type illustrated in **Figure 3.1**.

The linearity of the composite calibration plot, with an intercept well within 3× the regression SD, and the overall SD of ~0.02% FFA indicate that the three individual calibrations that were performed gave highly consistent results. However, it was noted that calibrations tended to drift over a period of a week, and this was found to be due to changes in the K-phthal reagent, making it necessary to perform a reagent blank correction. Although K-phthal is practically insoluble in 1-PrOH and is delivered as a suspension, small amounts do solubilize slowly over time. Because the solubilized K-phthal has absorptions that overlap with the ν (COO^-) band used to quantitate the FFA salts, it can introduce a measurable bias into FFA measurements over time, albeit taking several weeks to develop in a freshly prepared reagent. To account for this reagent

background signal, a mineral oil, which does not contain any FFA, is run as a blank to compensate for absorptions contributed by any solubilized K-phthal. The apparent FFA contribution of the blank is subtracted from the values obtained for all oil samples subsequently analyzed to account for any changes in K-phthal concentration.

Duplicate analyses of calibration standards were conducted 24 h apart to confirm that K-phthal does not attack TAG. Based on the MD_r and SDD_r of 017% and 0.024%, respectively, it was concluded that no significant saponification took place over that time period. This finding was corroborated by similar studies with samples of various refined oils.

3.4.3. Evaluation and Validation

To establish the efficacy of the methodology developed, standard addition experiments were carried out by adding a pre-prepared mixture of FFA obtained from olive oil to both refined corn and soybean oils, which were then analyzed in triplicate by the FTIR and the titrimetric AOCS method. The data obtained using these two methods are summarized in **Table 3.1**, with the accuracy of both methods being assessed by using the gravimetrically added amounts of FFA as the reference values.

In terms of overall reproducibility, the FTIR method had a mean SD_r of 0.029%, slightly better than that of the AOCS procedure (0.038%). In terms of overall accuracy relative to gravimetric standard addition, both methods have small positive biases, slightly greater than the overall SD_r, indicating that both the soybean and corn oils contained traces of FFA prior to standard addition. For each method, the value of SDD_a, which is a measure of the variability around the MD_a, is of the same order of magnitude as the SD_r value, with the FTIR method again performing slightly better. **Figure 3.4** present a composite plot of all the FFA results obtained for both oils by both methods against the reference gravimetric data. The regression equation for the composite data illustrated in **Figure 3.4** is:

$$FFA_{(IR/Titration)} = 0.99996\ FFA_{(Standard\ Addition)} + 0.047$$

$$R^2 = 0.999 \qquad SD = 0.030 \qquad [3.2]$$

Table 3.1. Results of Triplicate Analyses of Oils Spiked with Various Amounts an FFA Mixture by the AOCS Titrimetric and FTIR Methods[a]

Oil	FFA spiked (% w/w)[b]	AOCS Method		FTIR Method	
		Mean	SD	Mean	SD
Soybean	0.000	0.028	0.003	0.015	0.032
	0.501	0.524	0.015	0.526	0.019
	1.002	1.063	0.034	1.042	0.035
	1.508	1.543	0.061	1.549	0.031
	2.003	2.033	0.029	2.056	0.032
	2.490	2.531	0.055	2.505	0.037
Corn	0.000	0.057	0.011	0.066	0.020
	0.500	0.546	0.009	0.573	0.027
	0.999	1.120	0.053	1.038	0.023
	1.475	1.573	0.068	1.512	0.005
	2.002	2.094	0.076	1.991	0.088
	2.502	2.578	0.051	2.548	0.002
Mean SD_r		0.038		0.029	
MD_a		0.059		0.036	
SDD_a		0.030		0.022	

[a]Abbreviations: SD_r, SD for reproducibility; MD_a, mean difference for accuracy; SDD_a, SD of the differences for accuracy.

[b]Expressed as % oleic acid.

The plot and regression equation illustrate the excellent concurrence between the titrimetric and FTIR methods as well as their comparable ability to track the amounts of FFA added gravimetrically to the two oils.

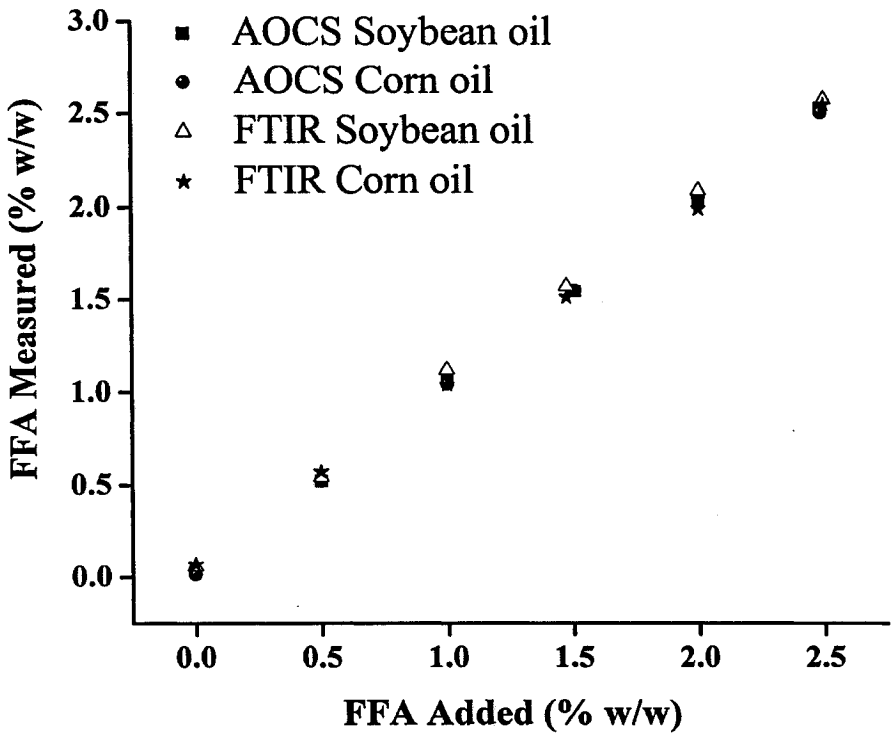


Figure 3.4. Graphical comparison of results obtained by the AOCS and FTIR methods for the oils that were subjected to standard addition of FFA mixture, relative to the gravimetrical data.

To further validate the performance of the FTIR method, five AOCS Smalley check samples were analyzed in triplicate. The results obtained relative to the analytical data provided with these oil samples are presented in **Table 3.2**. In general, there is excellent concurrence between the FTIR mean and Smalley mean FFA values except for crude coconut oil. Analysis of the Smalley samples in our laboratory by the AOCS method were also in line with the Smalley means, except again for crude coconut oil, which produced a value of 0.312 ± 0.005, very much in line with the FTIR result obtained, suggesting that the FFA content of this sample had changed in the time that had elapsed since its analysis in laboratories participating in the Smalley check sample program. Considering the results for the Smalley check samples as well as those obtained by standard addition, it is evident that the FTIR method is capable of producing accurate and reproducible FFA data independent of oil type and appears to be a valid alternative to the AOCS titrimetric procedure.

Table 3.2. Results of Triplicate Analysis of Smalley Check Samples by AOCS Method and FTIR Method[a]

Oil sample	AOCS Method					FTIR Method	
	Max	Min	Mean	SD	n[b]	Mean	SD
Crude safflower oil	0.500	0.330	0.457	0.036	18	0.467	0.057
Cottonseed oil	0.180	0.040	0.130	0.053	8	0.156	0.058
Crude coconut oil	0.200	0.080	0.136	0.029	20	0.297	0.014
Crude corn oil	1.720	1.120	1.419	0.124	19	1.431	0.019
Marine oil	1.970	1.800	1.864	0.044	12	1.810	0.024

[a] Results expressed as % oleic acid (w/w)

[b] n = number of laboratories that reported results for each sample.

The FTIR methodology for FFA analysis developed in this work combines the respective advantages of the direct and indirect approaches previously described in the literature and overcomes their limitations. As in other indirect approaches, enhanced sensitivity by comparison with direct measurement of the FFA ν (C=O) band is achieved by reacting the FFA with a base and measuring the ν (COO^-) band of the salt formed. However, a key difference is the use of a K-phthal suspension in 1-PrOH as a reagent instead of the KOH/methanol reagent previously used **(6,10)** as its weakly basic properties provide a means of avoiding saponification. Furthermore, the extraction step previously required in the indirect FFA methods is eliminated because no phase separation occurs and the FFA salts remain soluble in the 1-PrOH/oil mixture. The use of differential spectroscopy provides a means of minimizing matrix effects by canceling out the spectral contributions of the oil and contaminants therein, and spectral interferences arising from the slight solubilization of K-phthal in a non-freshly prepared reagent are compensated for by performing a reagent blank correction. These combined procedural elements allow a calibration to be developed by utilizing gravimetrically prepared standards of hexanoic acid in oil. The net result is that the FTIR method developed is a *primary method*, independent of other methods for calibration. It is also rapid and simple

to execute, and the FTIR portion of the analysis has been automated by integrating the spectrometer with an autosampler (Thermal-Lube Inc., Pointe-Claire, QC, Canada), allowing for the analysis of up to 60 paired samples per hour. Given that only measurements at two wavelengths are needed for this analytical procedure, a simple dual-wavelength filter-based IR instrument could also be used. With these hallmarks of a sound general method, this new methodology could serve as the basis for an AOCS instrumental method for the analysis of FFA in edible oils.

3.5. ACKNOWLEDGMENT

The authors would like to thank Sultan Qaboos University, Oman, for support of Mr. Alawi and the Natural Sciences and Engineering Research Council (NSERC) of Canada for financial support of this research. The technical assistance of Ms. Claudia Mucciardi is also acknowledged.

3.6. REFERENCES

1. van de Voort, F. R., Sedman J. and Russin, T. Lipid Analysis by Vibrational Spectroscopy, *Eur. J. Lipid Sci. Technol.* 103, 815-826 (2001).
2. O'Brien, R. D. *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing Company, Lancaster, PA, 1998.
3. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., 1989, Champaign, AOCS Official Method Ca-5a-40.
4. Determination of the Free Fatty Acids in European Commission Regulation (EED) No. 2568/91, *Official Journal of the European Communities*, No. L 248 (5.9.91), p. 6.
5. Lanser, A. C., List, G. R., Holloway, R. K., and Mounts, T. L. FTIR Estimation of Free Fatty Acid Content in Crude Oils Extracted from Damaged Soybeans, *J. Am. Oil Chem. Soc.*, 68, 448-449 (1991).
6. Ismail, A. A., van de Voort, F. R. and Sedman, J. Rapid Quantitative Determination of Free Fatty Acids in Fats and Oils by FTIR Spectroscopy. *J. Am. Oil Chem. Soc.*, 70, 335-341 (1993).
7. Verleyen, T., Verhe, R., Cano, A., Huyghebaert, A. and De Greyt, W. Influence of Triacylglycerol Characteristics on the Determination of Free Fatty Acids in Vegetable Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 78, 981-984 (2001).
8. Man, Y. B., Moh, M. H. and van de Voort, F. R. Determination of Free Fatty Acids in Crude Palm Oil and Refined-Bleached-Deodorized Palm Olein Using

Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 76, 485-490 (1999).

9. Bertran, E., Blanco, M., Coello, J., Iturriaga, H., Maspoch, S. and Montoliu, I. Determination of Olive Oil Free Fatty Acid by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 76, 611-616 (1999).

10. Cañada, M. J. A., Medina, A. R. and Lendl, B. Determination of Free Fatty Acids in Edible Oils by Continuous-Flow Analysis with FT-IR Spectroscopic Detection, *Appl. Spectrosc.*, 55, 356-360 (2001).

11. van de Voort, F. R., Sedman, J., Yaylayan, V. and Saint Laurent, C. The Determination of Acid Number and Base Number in Lubricants by FTIR Spectroscopy. *Appl. Spectrosc.*, 57, 1425-1431 (2003).

12. Kishore N. R. A., *Guide to ASTM Test Methods for the Analysis of Petroleum Products and Lubricants* (ASTM, West Conshohocken, PA, 2000), p. 45.

13. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th edn., Arlington, 2000, Method 972.28.

BRIDGE

In Chapter 3, an FTIR method for determination of FFA content in edible oils was developed by solubilizing oils in 1-PrOH containing a weak base and spectrally evaluating the FFA salts formed, using differential spectroscopy to minimize matrix effects. The method focused on finding ways to avoid or eliminate problems that were not overcome by previously developed FTIR methods, such as saponification and calibration dependency on oil type. Although successful in achieving these goals and providing a reliable and reproducible instrumental method for the determination of FFA, this new FTIR method is not significantly better than standard titrimetric procedures in terms of sensitivity. In Chapter 4, the knowledge and experience gained from this initial work is used to develop a much more sensitive method which allows for the analysis of low levels of FFA in refined edible oils.

CHAPTER 4

A NEW METHOD FOR THE ANALYSIS OF LOW LEVELS OF FFA IN REFINED EDIBLE OILS

4.1. ABSTRACT

This paper summarizes the application of stoichiometric analytical approaches to quantitative IR analysis and describes the development of a rapid and sensitive Fourier transform infrared (FTIR) method using such an approach for the determination of low levels (<0.005%) of free fatty acids (FFA) in refined edible oils. The method simply involves mixing the sample with methanol containing 2 g/L sodium hydrogen cyanamide (NaHNCN) on a vortex mixer for 30 s to convert the FFA to their salts, centrifuging the sample to separate the methanol phase containing the FFA salts from the oil, recording the FTIR spectrum of the upper methanol layer in a 100 μm CaF_2 transmission flow cell, and ratioing this spectrum against that of the NaHNCN/methanol solution. The concentration of FFA salts is determined from the resulting differential spectrum by measurement of the ν (COO^-) absorbance at 1573 cm^{-1} relative to a reference wavelength of 1820 cm^{-1}. A calibration spanning the range 0-0.1% FFA (expressed as oleic acid) was devised by gravimetric addition of a defined, pure fatty acid to an acid-free oil. Validation of the method by standard addition of palmitic acid to a variety of oils yielded an overall standard error of <±0.001% FFA. Comparison of triplicate FTIR and IUPAC titrimetric analyses of oils spiked with palmitic acid demonstrated that this FTIR method was more sensitive, accurate and reproducible than the titration procedure, the latter having a significant positive bias of ~0.02%. Solvent/oil consumption in the FTIR method is 2 mL/10 g *vs.* 150 mL/20 g for the titrimetric procedure. The FTIR method developed is particularly well suited for the determination of the low levels of FFA in refined oils but can readily be adapted with a simple adjustment of the oil:methanol ratio to cover FFA levels of up to 4.0%.

4.2. INTRODUCTION

The application of chemometric techniques has represented a major advance in quantitative IR analysis. In particular, partial-least-squares regression (PLS) has been of

widespread utility (1) as it provides a powerful means of extracting quantitative spectral information related to component(s) of interest from the spectra of complex samples by mathematically modeling partially overlapping bands or other sources of spectral interference. However, as the complexity of the system increases, so does the difficulty of developing reliable PLS calibration models and we have encountered diverse circumstances where PLS proved to be unsatisfactory as a calibration technique. Consequently, an alternative approach to quantitative IR analysis was developed based on the use of a reagent that reacts stoichiometrically with the component(s) of interest to produce a readily measurable IR signal. Furthermore, by combining this "signal transduction" approach with differential spectroscopy, quantitation can often be achieved via a simple univariate calibration.

Table 4.1 illustrates a number of successful applications of this approach. In the first case, difficulties were encountered in developing a robust PLS calibration model for determination of the peroxide value (PV) of edible oils. PV is a measure of hydroperoxides (ROOH), the primary oxidation products in edible oils, but FTIR quantitation of these species was complicated by both spectral overlap and hydrogen-bonding interactions with a large variety of secondary oxidation products and other components that may be present in edible oils **(2)**. These problems were overcome by taking advantage of the rapid stoichiometric reaction of hydroperoxides with triphenylphosphine (TPP) to form triphenylphosphine oxide (TPPO), which has an isolated and intense absorption band that allowed for the accurate determination of PV down to <1.0 mequivalents ROOH/kg oil **(3)**.

The next three examples in **Table 4.1** concern the analysis of new and used lubricating oils, which is beset by even more complex matrix effects than those encountered in oxidized edible oils. In the case of moisture analysis **(4)**, signal transduction was carried out by stoichiometrically converting dimethoxypropane through its reaction with water into acetone, a strong IR absorber, providing an alternative to the problematic Karl Fischer titration procedure. For the determination of acid number (AN) and base number (BN) in lubricants, there was the additional complication of the "structural specificity" of IR analysis as opposed to the "chemical specificity" of the traditional titrimetric methods employed for these analyses. The latter methods directly

Table 4.1. FTIR Analyses Based on the Use of Stoichiometric Reactions for "Signal Transduction"

Analysis[a]	Reagent	Stoichiometric Reaction	FTIR Measurement	Ref.
PV (>1.0) **Edible Oils**	Triphenylphosphine (TPP) in 1-hexanol	ROOH + TPP → ROH + TPPO	542 cm^{-1} [X-sensitive ring-breathing vibration of phenyl groups in TPPO]	(2)
H_2O (<1000 ppm) **Lubricants**	1,3-Dimethoxypropane (DMP) in isooctane	$2H_2O + DMP \rightarrow CH_3COCH_3 + 2CH_3OH + H_2O$	1717 cm^{-1} [ν(C=O) absorption of acetone]	(3)
AN (<0.1 mg KOH/g) **Lubricants**	Potassium phthalimide (K-Phthal) in 1-PrOH	$HA + K^+Phthal^- \rightarrow Ph + A^-K^+$	1774 or 1727 cm^{-1} [ν(C=O) absorptions of phthalimide]	(4)
BN (<0.1 mg KOH/g) **Lubricants**	Trifluoroacaetic acid (TFA) in 1-PrOH	$B: + TFA \rightarrow TFA^-BH^+$	1679 cm^{-1} [ν(COO^-) absorption of trifluoroacetate]	(5)
FFA (>0.2% C 18:1) **Edible Oils**	Potassium phthalimide (K-Phthal) in 1-PrOH	$RCOOH + K^+Phthal^- \rightarrow RCOO^- + Ph$	1570 cm^{-1} [ν(COO^-) absorption of $RCOO^-$] *or* 1776 cm^{-1} [ν(C=O) absorption of phthalimide]	(6)
FFA(>0.002% C 18:1) **Refined Edible Oils**	Sodium hydrogen cyanamide in methanol	$RCOOH + NaHNCN \rightarrow RCOO^- + H_2NCN$	1573 cm-^{1} in spectrum of methanol extract [ν(COO^-) absorption of $RCOO^-$]	This work

[a]PV = Peroxide Value, H_2O = Moisture, AN = Acid Number, BN = Base Number, FFA = Free Fatty Acids.

measure a large variety of acids and bases, their response being dependent only on the pK_a of the acid or base in relation to the titrimetric endpoint. In contrast, prediction of AN or BN by direct IR analysis would require the development of calibrations that model all the species contributing to the acidic/basic characteristics of lubricating oils, many of which are undefined. This severe limitation was overcome by reacting all the acidic or all the basic species with, respectively, a basic or an acidic "signal-transducing" reagent. By subtracting the spectrum of the unreacted sample from that of the reacted sample, the spectral changes associated with the acid-base reaction were isolated, and the extent of conversion of the "signal-transducing" reagent could be directly measured to determine the AN or BN of the sample **(5)**.

The last two examples cited in **Table 4.1** concern the application of similar concepts to the determination of the free fatty acid (FFA) content of edible oils, the first of which was described in a previous paper **(6)**. Among the oil quality parameters, FFA content is a crucial factor associated with the quality and economic value of edible oils, especially for unrefined high value oils such as olive oil. FFA content is also an important quality indicator in relation to oil processing and is used to assess deodorizer efficiency or as an indicator of frying oil quality **(7,8)**. FFA content is most commonly determined by titration of an oil, dissolved in neutralized ethanol or ethanol/diethyl ether, with a strong base to a phenolphthalein endpoint **(9,10)**. Although the standardized titrimetric methods are fairly sensitive, with limits of detection (expressed as percent oleic acid) on the order of 0.03% being attainable, more sensitive methods would be useful for the analysis of refined, bleached and deodorized (RBD) oils, which tend to have FFA levels of ≤0.05% **(7)**, and could also provide an alternative means of monitoring secondary oxidation products **(11)** accumulating in an oil in the form of carboxylic acids. In recent years, a variety of approaches have been investigated as possible alternatives to the titrimetric methods employed to determine the FFA content of oils, including the use of flow injection systems **(12,13)** pH metric, potentiometric and colorimetric **(14-16)** methods, and chromatographic procedures **(17-20)** as well as FTIR-based spectroscopic techniques **(21-28)**. Although many of these offer substantial benefits, in terms of speed of analysis, amenability to automation, and/or a reduction in the use of solvents and the attendant environmental problems and disposal costs, none of them provide a substantive

gain in sensitivity over that attained with titrimetric methods. This paper describes a simple, robust FTIR method based on the concepts outlined above that is capable of measuring FFA levels as low as 0.005% in refined oils. Thus, the new methodology described in this paper exemplifies the means by which the sensitivity of IR analysis can be substantially enhanced under certain circumstances by the signal transduction/differential spectroscopy approach.

4.3. METHODOLOGY

Reagents and Standard Methods. Sodium hydrogen cyanamide (NaHNCN, 99+%), palmitic acid (99%), and anhydrous methanol (MeOH) were obtained from Aldrich (St. Louis, MO) and were all of analytical grade. Refined edible oils were purchased locally or obtained from Canamera Foods (Toronto, ON, Canada). The reagent solution employed in the FTIR FFA analysis was prepared by dissolving NaHNCN in anhydrous MeOH (2 g/L). This solution was allowed to stand for ~4 days or until the ν (C≡N) band at 2100 cm^{-1} completely disappeared before use.

Instrumentation. The FTIR spectrometer used for this study was a Bomem WorkIR (Bomem, Quebec, PQ, Canada) equipped with a DTGS detector and purged with dry air using a Balston dryer (Balston, Lexington, MA). The sample-handling accessory was a valved 100 μm CaF_2 transmission flow cell (Dwight Analytical, Toronto, ON, Canada). Samples were aspirated into the cell under vacuum, and the cell was flushed clean after each sample with 1 mL of methanol. All spectra were collected by co-adding 32 scans at a resolution of 8 cm^{-1} and a gain of 1.0. The spectrometer was controlled by an IBM-compatible Pentium 150-MHz PC running under proprietary Windows-based UMPIRE® (Universal Method Platform for InfraRed Evaluation) software (Thermal-Lube, Pointe-Claire, PQ, Canada). This software provides programming capabilities so that repetitive operations can be performed in a specified sequence and designated spectral data collected and processed through a calibration equation, thereby automating the analysis to provide direct output of FFA data.

Preparation of Calibration Standards. A series of 12 standards covering a range of 0-0.1% FFA was prepared by gravimetric addition of palmitic acid to a refined and deodorized soybean oil after it had been run though an activated silica gel column to

remove any traces of FFA and other oxygenated compounds. The FFA contents of the standards were expressed in terms of % oleic acid.

Sample Preparation. Ten grams (± 0.001 g) of each oil sample or standard was weighed on an analytical balance into a tared 15-mL clinical centrifuge tube. To the tube containing the oil, 2 mL of the NaHNCN reagent solution was added using a calibrated re-pipette. The tubes were capped, shaken on a vortex mixer for 30 s, and then centrifuged for 5 min at 6000 rpm (~5000×*g*) to ensure consistent separation between the oil and methanol phases.

Analytical Protocol. Approximately 1 ml of the methanol reagent was loaded into the transmission flow cell and its single-beam spectrum recorded to serve as the background spectrum. A new background spectrum was collected in the same manner after every 20 samples or 1 h, whichever occurred first. For both samples and calibration standards, 1 mL of the upper methanol layer formed after centrifugation was loaded into the cell and its single-beam spectrum was recorded and ratioed against the NaHNCN/MeOH background spectrum. The peak height of the carboxylate band at 1573 cm^{-1} was then measured relative to an invariant baseline point at 1820 cm^{-1}. The overall sample preparation procedure and analytical protocol is illustrated in **Figure 4.1**.

Calibration and Validation. The calibration standards were taken through the analytical protocol described above, and a calibration equation for the prediction of FFA content was derived by plotting the concentrations of the standards (% oleic acid) *vs.* carboxylate peak height. The reproducibility and accuracy of the FTIR method were assessed by standard addition, spiking three different acid-free oils (canola, soybean and sunflower) with known amounts of palmitic acid (w/w). These oils were each analyzed in triplicate, on different days, by both the IUPAC titrimetric method **(9)** and the FTIR method to allow a direct comparison of their performance. A comparative analysis of locally purchased refined oils (soybean, sunflower, peanut, and corn oils and a commercial oil blend) was also carried out.

4.4. RESULTS AND DISCUSSION

The various approaches that have been investigated for the determination of FFA content in edible oils by FTIR spectroscopy were reviewed in a previous paper **(6)**, and

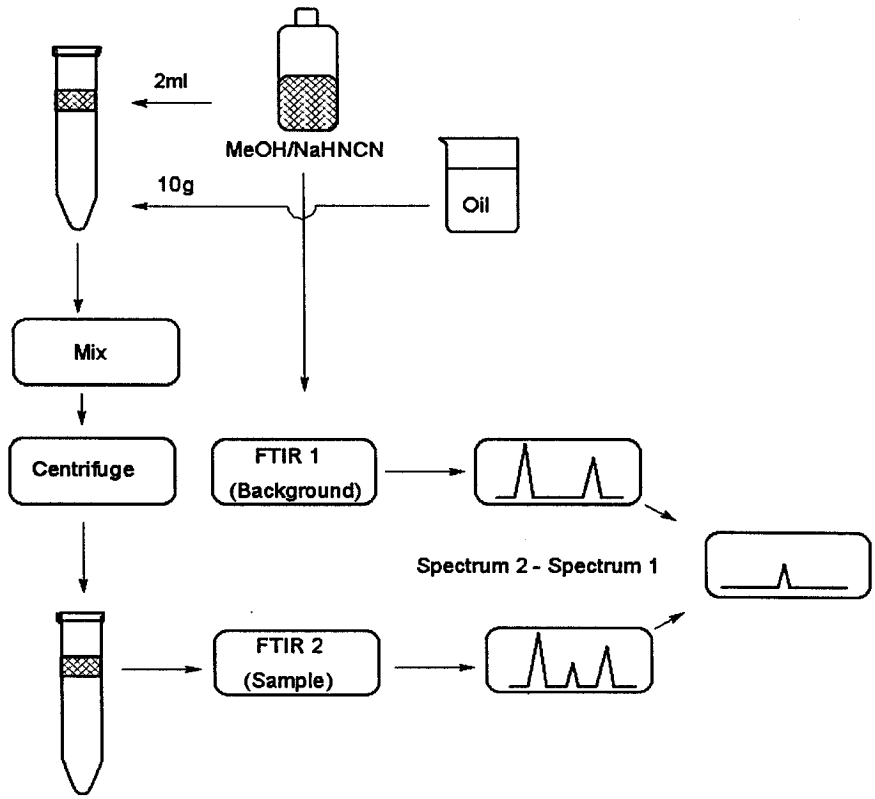


Figure 4.1. Schematic diagram of the sample preparation and analytical protocol.

the limitations of each approach discussed. In that paper, these limitations were addressed by the development of a new FTIR method based on previous work on AN determination in lubricants, which employed the mild base potassium phthalimide as a signal-transducing reagent in conjunction with differential spectroscopy to circumvent matrix effects **(6)**. Although the phthalimide FTIR procedure is both more accurate and reproducible than conventional titration, it is ultimately not more sensitive per se. This is largely due to the use of propanol as a solvent and polarity enhancer for the reaction, effectively diluting the COO^- IR signal. A means by which sensitivity could be improved would be to treat the oil with methanol containing a base which is immiscible with the oil to facilitate the acid-base reaction as well as concentrate the FFA salts in the methanol layer **(14)**. Such a procedure would have the additional advantage of minimizing matrix effects by partitioning out the spectral contribution of the oil. For highly accurate analyses, a weak base would be required to avoid any saponification of the oil and one

could use either the spectral changes associated with the loss of the base or formation of FFA salts as a basis for quantitation. Examination of a range of reagents led to the consideration of the sodium salt of carbodiimide (NaHNCN), which has a strong ν (C≡N) absorption at 2100 cm^{-1}, is readily soluble in methanol, and is capable of converting FFAs to their carboxylate salts but not capable of saponifying triacylglycerols. In the first instance, the proportionate decrease in the intensity of the ν (C≡N) band appeared to be a very good measure of the amount of FFAs spiked into oils. Although this reaction worked, its was found that the reagent spectrum was unstable and that the band at 2100 cm^{-1} slowly disappeared over a period of ~ four days with the concomitant appearance of two new bands at 1650 and 1610 cm^{-1} (**Figure 4.2**). Based on assignment of these two bands to C=N stretching and NH bending vibrations, respectively, the structural rearrangement from NaHN-C≡N → NaN=C=NH was postulated to be taking place in solution over time. This conversion was confirmed spectrally by dissolving NaHNCN in methyl alcohol-*d* (MeOD), whereby the band at 1610 cm^{-1} shifted about 100 cm^{-1} to lower frequency owing to hydrogen-deuterium exchange.

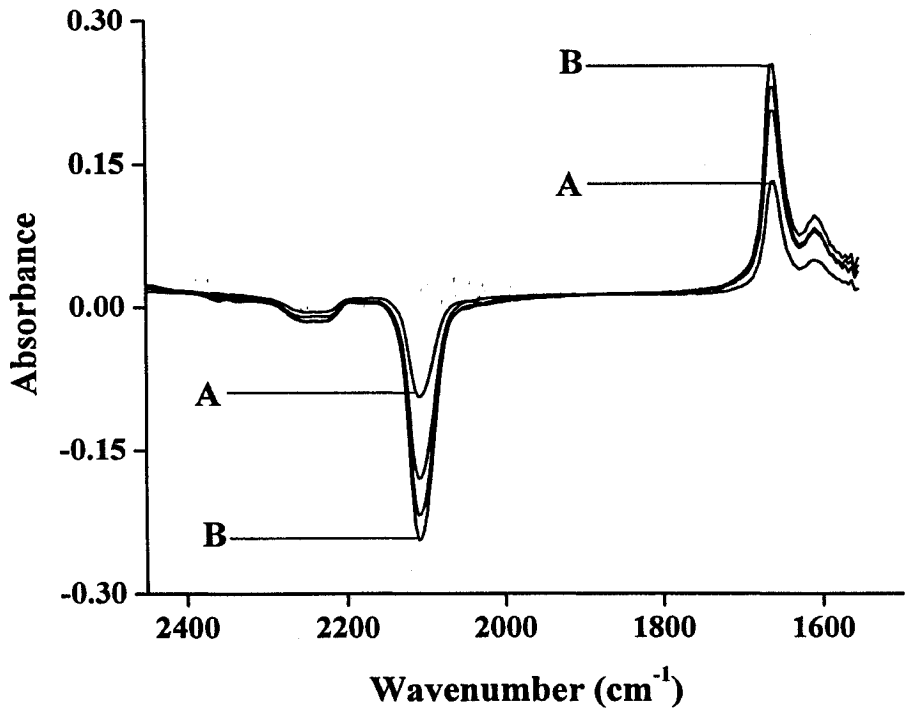


Figure 4.2. Differential spectra for sodium hydrogen cyanamide in methanol. The spectra show the decomposition of the reagent over time. A = one day; B = four days.

This molecular rearrangement, which was established to be complete within 4 days after preparation of the MeOH/NaHNCN reagent, did not affect its ability to convert FFAs to their respective salts without causing oil saponification. The reactivity of the converted MeOH/NaHNCN solution as well as its spectral characteristics remained stable, with solutions kept at room temperature being used for up to two months without any apparent deterioration in their efficacy. On the other hand, the measurement of the C≡N band originally envisioned as a basis for quantitation is lost as a result of this transformation. However, measurements made using the carboxylate band of FFA salts at 1573 cm^{-1} were both reproducible and responsive to low FFA levels because of the concentration of the FFA salts in the methanol layer and the high extinction coefficient of the carboxylate band. It was found that optimum sensitivity and reproducibility were achieved with a 5:1 oil:methanol ratio (10 g oil plus 2 mL of "aged" MeOH/NaHNCN reagent mixed in a standard 15-mL clinical centrifuge tube), producing consistent reactions and reproducible separations of the MeOH and oil phases when centrifuged for 5 min at 5000 × g. Oil stability with respect to saponification by the reagent was assessed by incubating oil samples with the reagent for 24 h and did not result in any measurable oil hydrolysis, but did lead to a minor displacement effect (~0.01% FFA) due to some additional oil migration into the methanol layer over extended periods of time.

4.4.1. Calibration

A calibration curve was developed by using a set of standards covering the range of 0.0 to 0.1% FFA, prepared by adding palmitic acid to acid-free soybean oil. **Figure 4.3** illustrates the differential spectra obtained for these standards using the optimized analytical protocol outlined in **Figure 4 1**. The carboxylate anion produced and extracted into the MeOH layer shows a rising absorbance at 1573 cm^{-1}, the other spectral features at 1650 cm^{-1} and the split band covering 1760-1700 cm^{-1} representing the ν (C=N) absorption of the base and the ν (C=O) ester linkage absorption of a small amount of solubilized oil. A plot of the absorbance measured at 1573 cm^{-1}, referenced to a single-point baseline at 1820 cm^{-1}, *vs.* % FFA (oleic acid) is presented in **Figure 4.4**. Linear regression of the data obtained resulted in the following relationship:

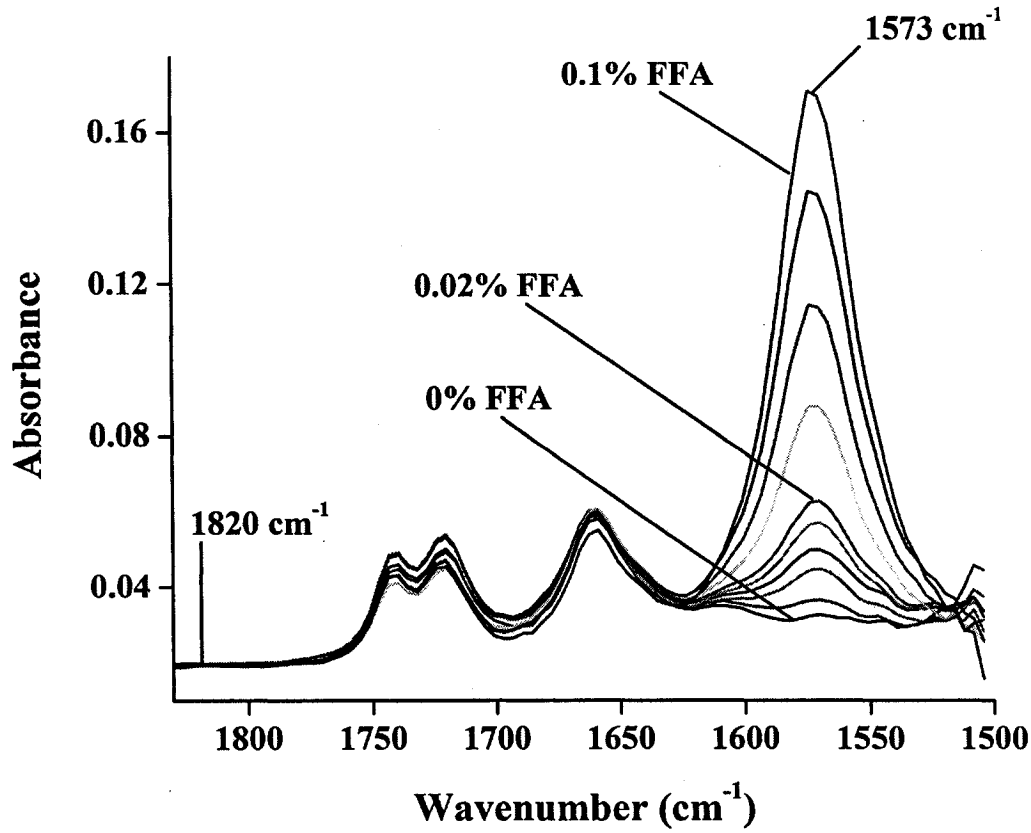


Figure 4.3. Differential spectra for soybean oil spiked with palmitic acid (0.0 – 0.1%) after carrying out the acid/base reaction. Spectra were recorded in a 100 μm cell at 8 cm^{-1} resolution.

$$\% \text{ FFA} = 0.70292\ A_{(1573/1820)} - 0.00883$$

$$R^2 = 0.9998; \quad \text{SD} = 6.706\times10^{-4} \qquad [4.1]$$

The regression SD implies that FFA levels in the order of $1/1000^{th}$ of a percent may be measurable by this technique.

4.4.2. Validation

Validation and comparison of the FTIR method relative to the IUPAC titrimetric method were carried out by analyzing three acid-free oils (soybean, canola and sunflower) spiked with palmitic acid. **Figure 4.5** presents comparative plots for triplicate

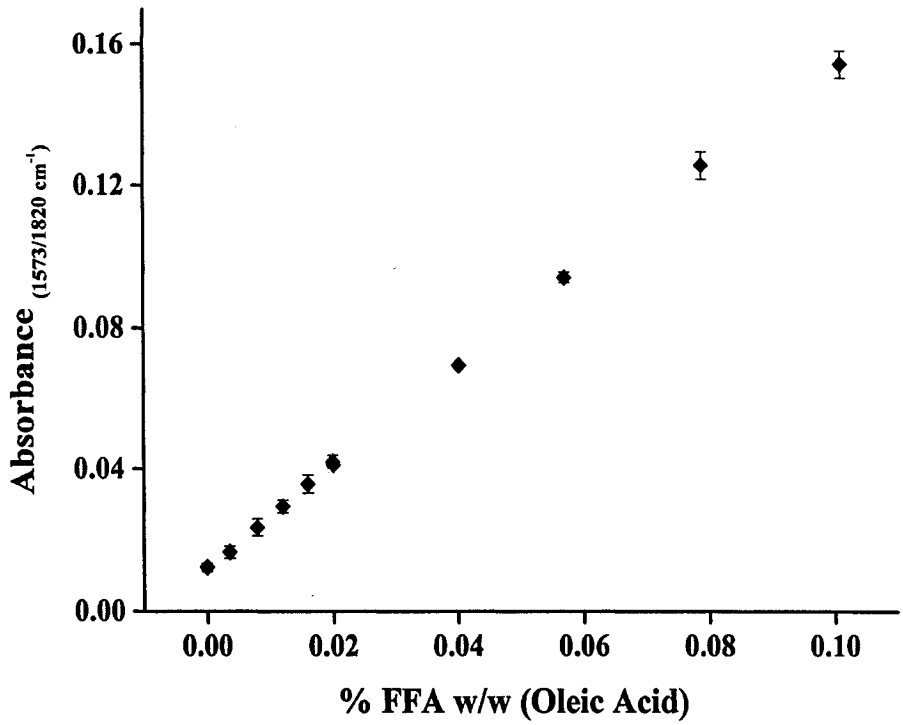


Figure 4.4. Calibration curve for %FFA in oil obtained from the differential spectra in Figure 4.2. The %FFA is expressed as % oleic acid (w/w). Error bar amplitude indicates mean ± SD of three replicates.

analyses of these oils by FTIR spectroscopy and the IUPAC titration procedure, respectively. The corresponding regression equations are:

$$FTIR_{FFA} = 1.004\ FFA + 4.4\times10^{-4}$$

$$R^2 = 0.9998 \qquad SD = 7\times10^{-4} \qquad [4.2]$$

$$IUPAC_{FFA} = 1.047\ FFA + 2.3\times10^{-2}$$

$$R^2 = 0.9887 \qquad SD = 5\times10^{-3} \qquad [4.3]$$

These results clearly indicate that the FTIR method tracks standard addition very well, with a slope and an intercept very close to unity and zero, respectively, with an overall error of about <0.001%. The titrimetric plot clearly shows greater variability.

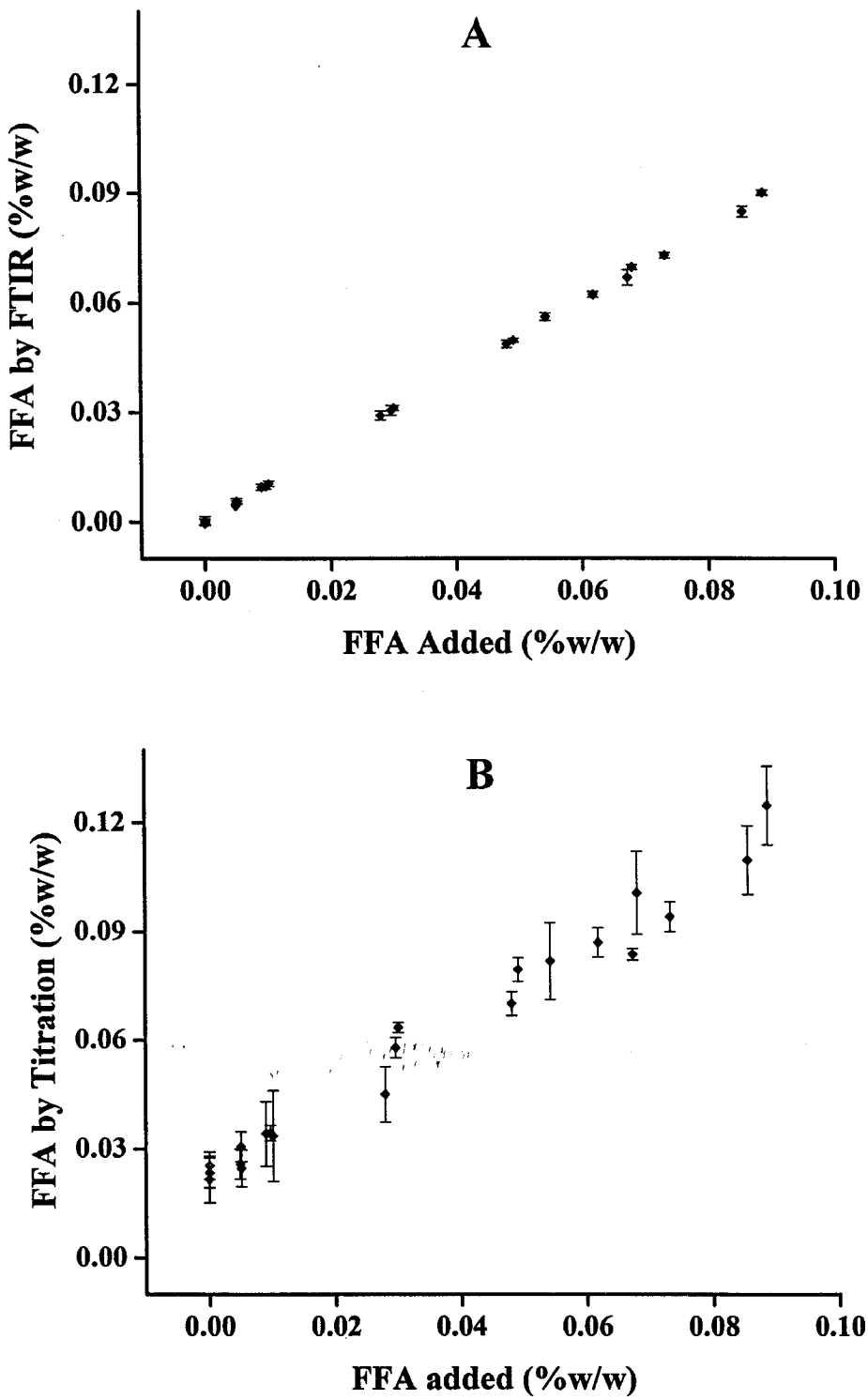


Figure 4.5. A plot of %FFA determined by the FTIR method (A) and %FFA determined by the titrimetric method (B) against the spiked amount. Error bar amplitude indicates mean ± SD of three replicates.

Table 4.2 presents the data in terms of the relative mean difference (MD) and standard deviation of the differences (SDD) for both accuracy (a) and reproducibility (r). The MDa of 0.025% for the titrimetric method indicates a significant bias relative to standard addition considering its SDDa, while the FTIR results are basically an order of magnitude better in terms of both accuracy and reproducibility with no significant bias. The apparent titrimetric over determination relative to the FTIR method may be due to systematic errors associated with a slower acid/base reaction owing to the lower solvent polarity, the substantive volume of solvent used, and CO_2 absorption, as well as variability contributed by the visual endpoint determination. **Table 4.3** presents comparative triplicate FTIR and titrimetric data obtained for locally purchased samples of refined oils. The two methods correlate (R^2 = 0.83), showing a similar trend as the standard addition data, with the titrimetric procedure predicting higher values individually as well as an overall mean bias of ~0.02% and yielding poorer reproducibility.

Table 4.2. Mean Difference (MD) and Standard Deviation of the Differences (SDD) for Accuracy ($_a$) for the Titrimetric and FTIR Procedures *vs*. Gravimetric Addition and for Reproducibility ($_r$) for Duplicate Analyses Carried Out by Titrimetric and FTIR Analyses, Respectively

Statistic	FTIR	Titration
MD_a	5.84×10^{-4}	2.50×10^{-2}
SDD_a	7.00×10^{-4}	5.10×10^{-3}
MD_r	4.56×10^{-4}	3.65×10^{-3}
SDD_r	9.47×10^{-4}	9.86×10^{-3}

The carbodiimide FTIR method was further examined in relation to expanding its general utility by changing its oil:reagent ratio. By decreasing the oil:reagent ratio from 5:1 to 1:4, while maintaining the same carbodiimide concentration, a calibration covering a range of 0-4% FFA was developed which produced the following calibration linear regression equation:

$$\% \text{ FFA} = 18.994 * A_{(1573/1820)} - 0.03598$$

$$R^2 = 0.9999 \qquad \text{SD} = 0.0206 \qquad [4.4]$$

Thus simply by changing oil:reagent ratio, semi-refined and crude oils can also be analyzed accurately using the same basic analytical protocol outlined in **Figure 4.1**. As such, the method developed provides a simple FTIR-based analytical procedure for the measurement of both very low and moderate levels of fatty acids in oils. Aside from providing excellent sensitivity and reproducibility, it overcomes many of the limitations associated with conventional titrimetric and potentiometric methods. There is no need to use an FTIR spectrometer for this analysis *per se*, given that measurements at only two wavelengths are required and a simple dual-wavelength filter instrument would suffice. In either case, the method is readily amenable to automation.

Table 4.3. Results of Triplicate Analyses of Locally Purchased Oil Samples by the IUPAC Reference (Titrimetric) Method and the FTIR Method

Oil type	Titrimetric method		FTIR method	
	% FFA(w/w)[a]	SD	% FFA(w/w)[a]	SD
Peanut oil	0.050	5.39×10^{-3}	0.030	7.03×10^{-5}
Sunflower oil	0.041	6.40×10^{-3}	0.021	9.43×10^{-4}
Corn oil	0.053	3.50×10^{-3}	0.024	4.39×10^{-4}
Mixed oil	0.061	7.15×10^{-3}	0.032	8.28×10^{-4}
Soybean oil	0.045	3.62×10^{-3}	0.020	1.62×10^{-4}
Overall Mean	0.050	--	0.026	--

[a] Expressed as % oleic acid

4.5. CONCLUSION

Our work on FTIR analysis of lubricating oils led us to develop a novel approach for the determination of acidity in non-aqueous systems based on the combined use of signal transduction via a stoichiometric reaction and differential spectroscopy. In subsequent work, we demonstrated the suitability of this approach for the quantitation of FFAs at levels of >0.2% oleic acid by employing the same reagent as used for the determination of acidity in lubricating oils. In the present study, the use of sodium carbodiimide and a modified procedure has allowed the analysis to be extended down to FFA levels as low as 0.001%, which would not be measurable by direct measurement of FFA absorptions in the IR spectra of oils. With a simple adjustment in the reagent:oil ratio the method becomes scalable and the analysis of semi-refined and crude oils containing up to 4.0% FFA is possible. The strength of the method is its sensitivity and allows for the possibility of using FFAs as an indicator of lipid oxidation and to more accurately monitor the refining processes carried out on edible oils.

4.6. ACKNOWLEDGMENT

F. R. van de Voort acknowledges the financial support of the Natural Sciences and Engineering Research Council of Canada and the cooperation of Canamera Foods for providing oil samples for analysis. A. Al-Alawi thanks Sultan Qaboos University for financial support for his PhD studies.

4.7. REFERENCES

1. Haaland, D. M. Quantitative Infrared Analysis of Borosilicate Films using Multivariate Statistical Methods. *Anal. Chem.*, 60, 1208-1217 (1988).
2. van de Voort, F. R., Ismail, A. A., Sedman, J., Dubois, J and Nicodemo, T. The Determination of Peroxide Value by Fourier Transform Infrared (FTIR) Spectroscopy. *J. Am. Oil Chem. Soc.*, 71, 921-926 (1994).
3. Ma, K., Van De Voort, F. R., Sedman, J. and Ismail, A. A. Stoichiometric Determination of Hydroperoxides in Fats and Oils by Fourier Transform Infrared Spectroscopy. *J. Am. Oil Chem. Soc.*, 74, 897-906 (1997).
4. van de Voort, F. R., Sedman, J., Yaylayan, V. and Saint Laurent, C. Determination of Acid Number and Base Number in Lubricants by Fourier Transform Infrared Spectroscopy. *Appl. Spectrosc.*, 57, 1425-1431 (2003).

5. van de Voort, F. R., Sedman, J., Yaylayan, V., Saint Laurent, C. and Mucciardi, C. Quantitative Determination of Moisture in Lubricants by Fourier Transform Infrared Spectroscopy. *Appl. Spectrosc.*, 58, 193-198 (2004).

6. Al-Alawi, A, van de Voort, F. R. and Sedman, J. New Method for the Quantitative Determination of Free Fatty Acids in Oil by FTIR Spectroscopy. *J. Am. Oil Chem. Soc.*, 81, 441-446 (2004).

7. O'Brien, R. D. *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing Company, Lancaster, PA, 1998

8. Senorans, F. J. and Ibanez; E. Analysis of Fatty Acids in Foods by Supercritical Fluid Chromatography. *Anal. Chim. Acta*, 465, 131-144 (2002).

9. International Union Pure and Applied Chemistry. *Standard Methods for the Analysis if Oils, Fats and Derivatives, 7th revised and enlarged edn.*, Paquot, C., and Hautfenne, A., Eds. Blackwell Scientific, Oxford, 1987

10. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., 1989, Champaign, IL. AOCS Official Method Ca-5a-40

11. Velasco, J., Andersen, M. L. and Skibsted, L. H. Evaluation of Oxidative Stability of Vegetable Oils by Monitoring the Tendency to Radical Formation. A Comparison of Electron Spin Resonance Spectroscopy with the Rancimat Method and Differential Scanning Calorimetry, *Food Chem.*, 85, 623-632 (2004).

12. Nouros, P. G., Georgiou, C. A. and Polissiou, M. G. Automated Flow Injection Spectrophotometric Non-Aqueous Titrimetric Determination of the Free Fatty Acid Content of Olive Oil. *Anal. Chim. Acta*, 351(1-3), 291-297 (1997).

13. Mariotti, E. and Mascini, M. Determination of Extra Virgin Olive Oil Acidity by FIA-Titration. *Food Chem.*, 73, 235-238 (2001).

14. Kwon, D. Y. and Rhee, J. S. A Simple and Rapid Colorimetric Method for Determination of Free Fatty Acids for Lipase Assay. *J. Am. Oil Chem. Soc.*, 63, 186-189 (1986).

15. Tur'yan, Ya. I., Berezin, O. Yu; Kuselman, I. and Shenhar, A. pH-Metric Determination of Acid Values in Vegetable Oils without Titration. *J. Am. Oil Chem. Soc.*, 73, 295-301 (1996).

16. Takamura, K., Fuse, T., Arai, K. and Kusu, F. A Review of a New Voltammetric Method for Determining Acids. *J. Electroanal. Chem.*, 468, 53-63 (1999).

17. Dermaux, A., Sandra, P. and Ferraz, V. Analysis of Free Fatty Acids and Fatty Acid Phenacyl Esters in Vegetable Oils and Margarine by Capillary Electro-Chromatography. *Electrophoresis*, 20, 74-79 (1999).

18. Kotani, A., Kusu, F. and Takamura, K. New Electrochemical Detection Method in High-Performance Liquid Chromatography for Determining Free Fatty Acids. *Anal. Chim. Acta*, 465, 199-206 (2002).

19. Rosenfeld, J. M. Application of Analytical Derivatizations to the Quantitative and Qualitative Determination of Fatty Acids. *Anal. Chim. Acta*, 465, 93-100 (2002).

20. Senorans, F. J. and Ibanez, E. Analysis of Fatty Acids in Foods by Supercritical Fluid Chromatography. *Anal. Chim. Acta*, 465, 131-144 (2002).

21. Lanser, A. C., List, G. R., Holloway, R. K. and Mounts, T. L. FTIR Estimation of Free Fatty Acid Content in Crude Oils Extracted from Damaged Soybeans, *J. Am. Oil Chem. Soc.*, 68, 448-449 (1991).

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

23. Bertran, E., Blanco, M., Coello, J., Iturriaga, H., Maspoch, S. and Montoliu, I. Determination of Olive Oil Free Fatty Acid by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 76, 611-616 (1999).

24. Man, Y. B., Moh, M. H. and van de Voort, F. R. Determination of Free Fatty Acids in Crude Palm Oil and Refined-Bleached-Deodorized Palm Olein Using Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 76, 485-490 (1999).

25. Verleyen, T., Verhe, R., Cano, A., Huyghebaert, A. and De Greyt, W. Influence of Triacylglycerol Characteristics on the Determination of Free Fatty Acids in Vegetable Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc.*, 78, 981-984 (2001).

26. Zhang, H-Z and Lee, T-C. Rapid Near-Infrared Spectroscopic Method for the Determination of Free Fatty Acid in Fish and Its Application in Fish Quality Assessment. *J. Agric. Food Chem.*, 45, 3515-3521 (1997).

27. Man, Y. B. C. and Moh, M. H. Determination of Free Fatty Acids in Palm Oil by Near-Infrared Reflectance Spectroscopy, *J. Am. Oil Chem. Soc.*, 75, 557-562 (1998).

28. Muik, B., Lendl, B., Molina-Diaz, A. and Ayora-Canada, M. J. Direct, Reagent-Free Determination of Free Fatty Acid Content in Olive Oil and Olives by Fourier Transform Raman Spectrometry, *Anal. Chim. Acta*, 487, 211-220 (2003).

29. Cañada, M., Medina, A. and Lendl, B. Determination of Free Fatty Acids in Edible Oils by Continuous-Flow Analysis with FT-IR Spectroscopic Detection, *Appl. Spectrosc.*, 55, 356-360 (2001).

BRIDGE

In Chapter 4, an approach to FFA analysis was developed based on the treatment of oils with an oil immiscible solvent containing a weak base to convert the FFA to their carboxylate salts and concentrate the salts in a small volume to increase the sensitivity of the method. By comparison with the method described in Chapter 3, this approach also provided the benefit of eliminating the need to prepare two samples to obtain one analytical result as well as allowing an overall simplification of the analysis. This extraction/concentration approach is considered in Chapter 5 as a possible means by which the moisture content of edible oils can be determined.

CHAPTER 5

A NEW FTIR METHOD FOR THE DETERMINATION OF MOISTURE IN EDIBLE OILS

5.1. ABSTRACT

A rapid, practical, and accurate FTIR method for the determination of moisture content in edible oils has been developed based on the extraction of water from oil samples into dry acetonitrile. A calibration curve covering a moisture content range of 0-2000 ppm was developed by recording the mid-IR spectra of moisture standards, prepared by gravimetric addition of water to acetonitrile that had been dried over molecular sieves, in a 500 μm ZnSe transmission flow cell and ratioing these spectra against that of the dry acetonitrile. Water was measured in the resulting differential spectra using either the OH stretching (3629 cm^{-1}) or bending (1631 cm^{-1}) bands to produce linear standard curves having SDs of ~ ±20 ppm. For moisture analysis in oils, the oil sample was mixed with dry acetonitrile in a 1:1 w/v ratio, and after centrifugation to separate the phases, the spectrum of the upper acetonitrile layer was collected and ratioed against the spectrum of the dry acetonitrile used for extraction. The method was validated by standard addition experiments with samples of various oil types, as well as with oil samples deliberately contaminated with alcohols, hydroperoxides, and free fatty acids to investigate possible interferences from minor constituents that may be present in oils and are potentially extractable into acetonitrile. The results of these experiments confirmed that the moisture content of edible oils can be assessed with high accuracy (on the order of ±10 ppm) by this method, thus providing an alternative to the conventional, but problematic Karl Fischer method and facilitating the routine analysis of edible oils for moisture content.

5.2. INTRODUCTION

Moisture content is an important parameter associated with the processing and quality of edible oils. Commonly used in processes such as degumming and refining, water is subsequently removed by centrifugation, adsorption or vacuum drying. Although water has limited solubility in fats and oils, the moisture content must be minimized for

the effective adsorption of remaining soap traces after refining and to reduce lipid hydrolysis during and after processing **(1)** Generally, the moisture content of refined oils should be <0.1% (1000 ppm) and preferably <500 ppm **(2)**. Methods for moisture analysis in edible oils that serve as Official Methods of the American Oil Chemists' Society (AOCS) **(3)** include evaporative, distillation or titrimetric procedures as well as the Karl Fischer (KF) method, which is based on the selective reaction of water with a mixture of iodine, a base, sulfur dioxide, and an alcohol followed by either titrimetric, potentiometric **(4,5)** or coulometric **(6,7)** quantitation. The coulometric KF method is considered to be the most accurate and sensitive method available and is the gold standard for the determination of a wide range of moisture contents (1-25,000 ppm). However, it is an exacting procedure, uses expensive and environmentally problematic reagents, and is affected by oil oxidation end products such as aldehydes, ketones, and hydroperoxides, as these can undergo aldol condensation and/or redox reactions under the conditions of the test **(8)**. Thus, there is effectively no simple, convenient, sensitive method available for the analysis of moisture in fats and oils.

At first glance, IR spectroscopy would appear to be an obvious instrumental approach for the direct measurement of moisture in edible oils, given the strong absorption bands of water in the mid-IR portion of the spectrum, **(9)** and a limited feasibility study of the determination of moisture content in crude palm oil by FTIR spectroscopy has recently been reported **(10)** However, a number of complicating factors that were not taken into account in the previous study must be considered in the development of a *generalized* FTIR method for the determination of moisture in edible oils. First of all, the band shapes and intensities of the water absorptions, particularly the OH stretching vibrations, are strongly affected by the extent of hydrogen bonding between water molecules, which will depend on the moisture content in an oil, as well as between water and other hydrogen-bonding constituents. Furthermore, OH-containing constituents that are frequently present in oils (e.g., alcohols, free fatty acids (FFAs), and/or hydroperoxides) not only perturb the water absorptions via hydrogen-bonding interactions but also give rise to spectrally interfering bands. In a recent publication **(11)**, we reported a new approach to the FTIR analysis of moisture in new and used mineral-based lubricants in which similar "matrix effects" were overcome by the combined use of

the stoichiometric reaction of water with dimethoxypropane (DMP) to produce acetone **(12,13)** and differential spectroscopic techniques to quantitate this end product by measurement of its *v* (C=O) absorption. This approach, however, is not applicable to edible oils because the triacylglycerol ester linkages give rise to an intense *v* (C=O) absorption that would completely mask the *v* (C=O) band of acetone in a transmission spectrum, except at extremely short path lengths. Moreover, the use of ATR to overcome this limitation is impractical owing to the difficulty of preventing the sample from picking up moisture from the environment, nor would it provide sufficient sensitivity for the analysis of moisture at the low levels present in edible oils. Hence, an alternative approach is required for edible oils, and this paper describes the evaluation of an approach based on the extraction of water from the oil into a suitable solvent, culminating in the development of an acetonitrile extraction method that allows for the accurate determination of low levels of moisture in edible oils.

5.3. METHODOLOGY

Reagents. HPLC grade acetonitrile, obtained from Fisher Scientific (St. Louis, MO), was kept over 4-8 mesh 4Å molecular sieves and dispensed using a re-pipette (Hirschmann-Laborgerate, Germany) protected by desiccant to prevent moisture ingress. Various refined edible oils were obtained locally, and some samples of these oils were spiked with constituents spectrally representative of contaminants found in edible oils; these included *tert*-butyl hydroperoxide, glycerol, and oleic acid, all obtained from Sigma-Aldrich.

Instrumentation. The FTIR spectrometer used for this study was a Bomem WorkIR (Bomem, Quebec City, PQ, Canada) equipped with a DTGS detector and purged with dry air using a Balston dryer (Balston, Lexington, MA). The spectrometer was controlled by an IBM-compatible Pentium 150-MHz PC running proprietary Windows-based UMPIRE® (Universal Method Platform for InfraRed Evaluation) software (Thermal-Lube, Pointe-Claire, PQ, Canada). A 500 μm ZnSe transmission flow cell (International Crystal Laboratories, Garfield, NJ) equipped with Luer fittings was employed for the handling of oil samples. As illustrated in **Figure 5.1**, the cell inlet was connected to a 10 cm, 18-gauge stainless steel aspiration needle via flexible silicone

tubing, and the outlet line was connected to vacuum via a trap and was fitted with a valve to allow both aspiration of the sample through the cell and emptying of the cell; in the latter process, the air entering the cell was passed through a desiccant tube containing molecular sieves and Drierite to prevent environmental moisture from contaminating the analytical system. All spectra were collected by co-adding 32 scans at a resolution of 8 cm^{-1} and a gain of 1.0.

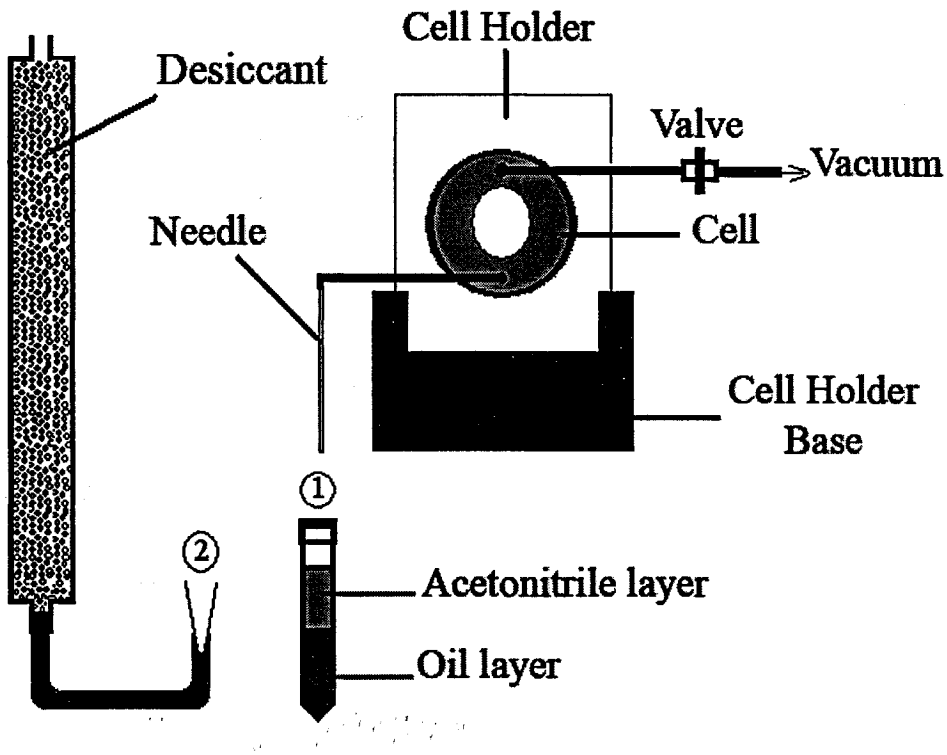


Figure 5.1. Schematic diagram of the FTIR sample handling system. To facilitate loading, the upper acetonitrile layer of the centrifuged or separated sample (1) is vacuum aspirated into the IR cell using the 10 cm stainless steel needle attached by flexible tubing to the IR cell. Between sample loadings, the needle is inserted into the desiccant tube outlet (2) to ensure that air used to flush the tubing and the cell does not introduce any moisture.

Analytical Protocol. The sample preparation procedure and analytical protocol developed for the determination of the moisture content of oils by extraction of the moisture into dried acetonitrile and its quantitation by FTIR spectroscopy are illustrated

in **Figure 5.2**. For the analysis of oil samples having moisture contents in the range of 50-2000 ppm, a 1:1 (v/w) acetonitrile:oil ratio was established to be suitable. Accordingly, 5 g of the sample was added to a tared 15-mL clinical centrifuge tube and weighed on an analytical balance, with the weight recorded to ±0.001 g, and 5 mL of the dried acetonitrile was then added to the tube using a calibrated re-pipette. The tubes were capped, shaken on a vortex mixer for 30 s, and then centrifuged for 2 min at ~6000 rpm (~5000×*g*) to separate the oil and acetonitrile phases. Alternatively, adequate separation of the phases could be achieved by letting the sample stand for ~10 min, eliminating the need for the centrifugation step. Approximately 2 mL of the upper acetonitrile layer was then aspirated into the transmission flow cell, and its spectrum recorded and ratioed against the spectrum of the dry acetonitrile used to extract moisture from the oil samples. A new acetonitrile background spectrum was collected after every 20 samples or after 1 h, whichever came first.

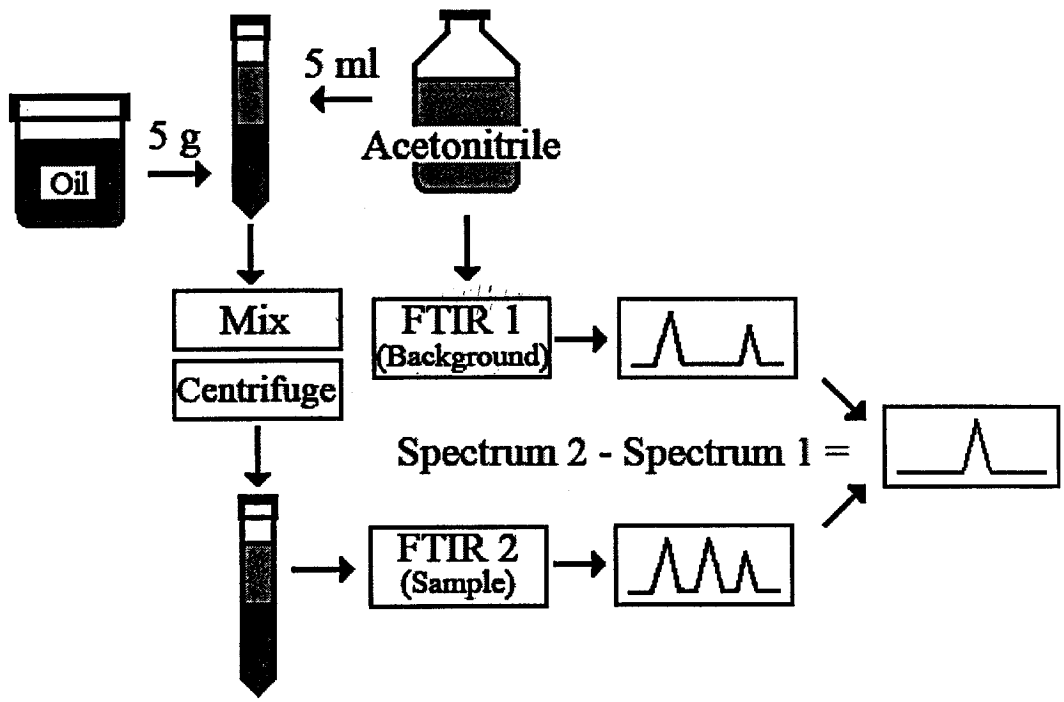


Figure 5.2. Schematic diagram of the sample preparation procedure for moisture analysis of edible oils and the FTIR spectral analytical protocol.

Calibration and Validation. Two calibration approaches were evaluated: one based on the use of primary water/acetonitrile standards and the other on water/oil standards. Twenty primary water/acetonitrile standards were prepared gravimetrically by adding distilled water to dry acetonitrile to cover a range of 0-1000 ppm, and a set of 12 water/oil standards (0-2000 ppm) were similarly prepared by gravimetrically adding distilled water to canola oil previously kept over molecular sieves for a minimum of 1 week. The primary standards were analyzed directly by FTIR spectroscopy, while the water/oil standards were taken through the analytical protocol illustrated in **Figure 5.2**. In both cases, calibration equations were developed relating the moisture added (in ppm) to the three measurable water bands at 3629, 3541, and 1631 cm^{-1}, relative to a baseline point at 2500 cm^{-1}. The reproducibility and accuracy of the FTIR method were assessed by standard addition of water to various oils (olive, corn, safflower, peanut, and sunflower), each of these samples being analyzed in triplicate, on different days, with the FTIR-predicted moisture contents being compared with the amounts added.

5.4. RESULTS AND DISCUSSION

5.4.1. General Considerations

The objective of this study was to develop a generally applicable method for the routine analysis of moisture in edible oils based on extraction of the moisture into a suitable solvent followed by quantitation by FTIR spectroscopy. The solvent initially considered was D_2O, in which each molecule of H_2O extracted from the oil sample would rapidly undergo hydrogen-deuterium exchange to produce two HOD molecules, thereby effectively doubling the spectral signal for each molecule of H_2O present. Thus, in principle, samples could be analyzed by mixing a defined excess of D_2O with the oil, allowing the oil and D_2O to separate, and quantitating the HOD formed by measuring the intensity of the characteristic HOD bands at 3375 and 1450 cm^{-1} in the spectrum of the D_2O layer. However, although this approach worked well for clean refined oils, the presence of OH-containing constituents in the oil (e.g., alcohols, hydroperoxides or FFAs), which also undergo hydrogen-deuterium exchange with D_2O, albeit more slowly, resulted in significant overestimation of moisture content, as evidenced by standard addition experiments. This problem, in conjunction with the relatively high cost of D_2O,

led us to seek another extraction solvent in which the concentration of H_2O could be accurately quantitated.

Among the solvents investigated, acetonitrile was ultimately selected. It was found to be sufficiently polar to be immiscible with edible oils and solubilize water while having limited capacity to solubilize the other potentially interfering OH-containing constituents that may be present in oils. Acetonitrile is also a highly suitable solvent in which to measure moisture levels by IR spectroscopy as it does not absorb strongly in the portions of the mid-IR spectrum where water absorbs. This lack of spectrally interfering bands makes it possible to use path lengths of up to 1000 μm for the analysis of low levels of moisture, thereby providing high sensitivity. Finally, in contrast to the spectrum of water itself, in which the two OH stretching absorptions are blended into one large diffuse band owing to the hydrogen-bonding network, the spectrum of water diluted in acetonitrile at mole fractions of <0.1 exhibits two discrete OH bands, which has been attributed to the binding of water molecules exclusively to acetonitrile rather than to each other at these high dilutions **(14)**. Thus, the spectra of water added to acetonitrile at levels of 400 and 800 ppm, presented in **Figure 5.3**, exhibit clearly delineated bands at 3629 and 3541 cm^{-1} together with the weaker HOH bending vibration at 1631 cm^{-1}.

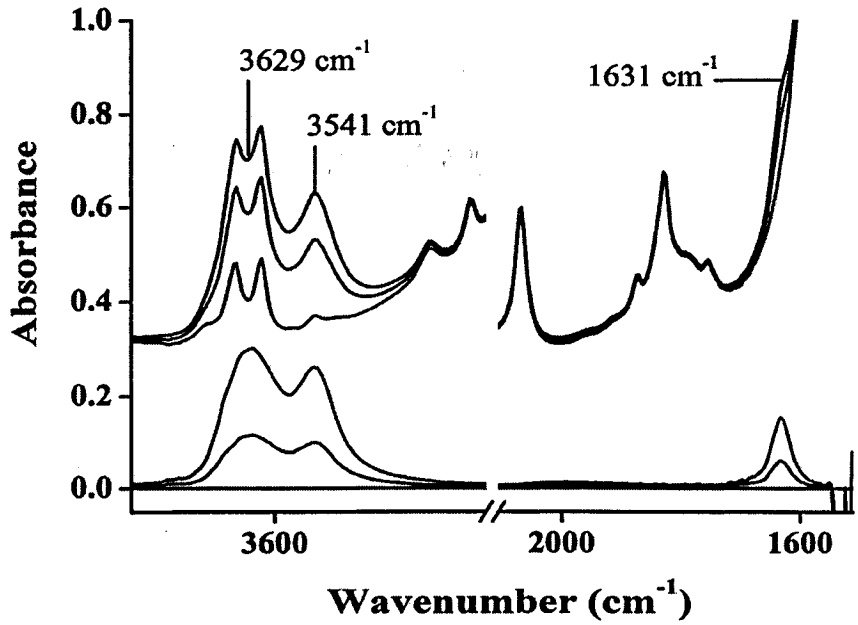


Figure 5.3. Spectra of water/acetonitrile standards (0, 400, and 800 ppm added water) (upper series) and the corresponding spectra obtained after subtraction of the spectrum of the acetonitrile employed to prepare the standards (lower series).

5.4.2. Calibration Development

Primary calibrations were devised using 20 standards prepared by addition of water to acetonitrile (dried over molecular sieves) to obtain moisture contents in the range of 0-1000 ppm based on the gravimetrically added amount. The contribution of any water present in the acetonitrile was not taken into account in calculating the moisture contents of these standards because the method was designed to employ differential spectra, obtained by subtraction of the spectrum of the acetonitrile used to prepare the standards from the spectrum of each standard, thereby eliminating the spectral contributions of any residual water in the dried acetonitrile.

The spectra of the standards were recorded in a 500 μm cell, and the following linear regression equations were obtained for the 3629, 3541, and 1631 cm^{-1} bands in the differential spectra:

$$H_2O\ (ppm) = 3420.82 * A_{3629\ cm^{-1}} - 4.29$$

$$R^2 = 0.999 \qquad SD = 17.7 \qquad [5.1]$$

$$H_2O\ (ppm) = 3934.92 * A_{3541\ cm^{-1}} - 5.55$$

$$R^2 = 0.999 \qquad SD = 17.4 \qquad [5.2]$$

$$H_2O\ (ppm) = 6941.50 * A_{1631\ cm^{-1}} + 2.33$$

$$R^2 = 0.999 \qquad SD = 16.9 \qquad [5.3]$$

These equations indicate relative sensitivities of ~3.4, 3.9, and 6.9 ppm per milliabsorbance unit for the 3629, 3541, and 1631 cm^{-1} bands, respectively, with SDs of <20 ppm.

Figure 5.4 presents a series of differential spectra obtained for a second set of standards (n = 12) prepared by spiking canola oil with water (0-2000 ppm) and processing the samples in accordance with the protocol illustrated in **Figure 5 2.** The calibration equations derived from these spectra were also linear, with similar slopes, correlation coefficients, and regression SDs as those obtained using the primary

water/acetonitrile standards, but had negative intercepts of ~280 ppm, indicating that the canola oil contained about 0.03% moisture. A notable difference between the differential spectra of the two sets of standards (**Figure 5.3** *vs.* **Figure 5.4**) is the presence of a carbonyl band (peak D) at 1740 cm^{-1} in the spectra of the set prepared by extraction of water from oil into acetonitrile. It was determined experimentally that the positions of the ν (C=O) absorptions of oils (triacylglycerol ester linkage) and FFA mixtures derived from the same oils were identical in acetonitrile. However, although FFAs are freely soluble in acetonitrile, it was considered unlikely that peak D was solely due to FFAs extracted from the oil, owing to their low levels in refined oils (<0.05%), and this was borne out by further examination of the spectra, in that peak D was not accompanied by any FFA absorption at ~3300 cm^{-1}. Thus, the observation of peak D was taken as an indication that some amount of oil was extracted into the acetonitrile layer.

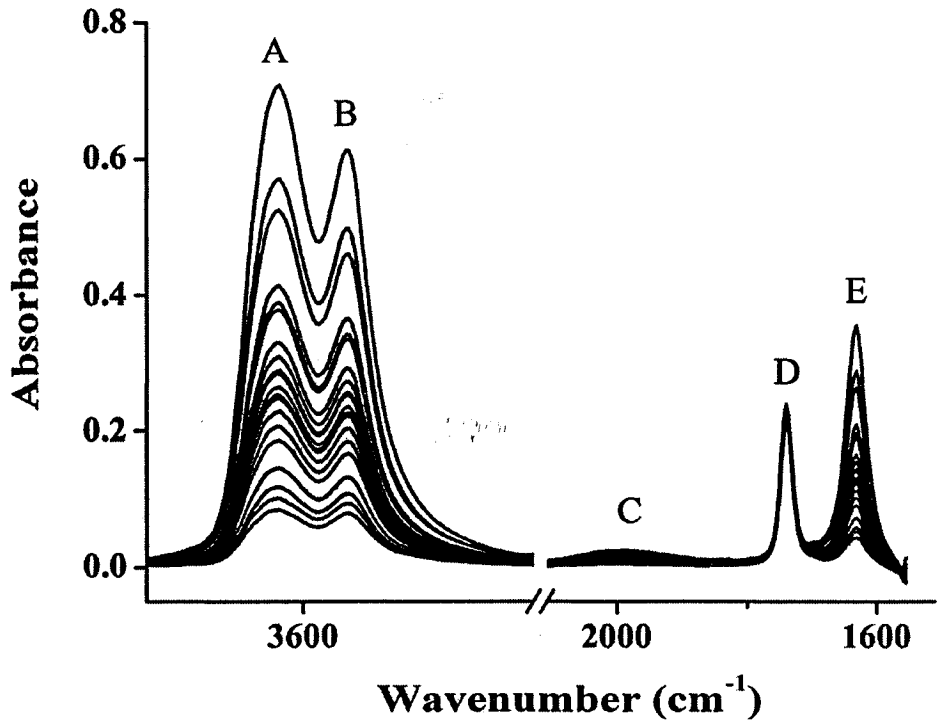


Figure 5.4. Differential spectra obtained after extraction of water from water/canola oil standards (0–2000 ppm water) into acetonitrile, recording the spectrum of the acetonitrile layer, and ratioing the spectrum against that of the acetonitrile extraction solvent. The major spectral features are identified as follows: A and B (3629 and 3541 cm^{-1}, respectively), OH stretching vibrations; C, water association band; D (1740 cm^{-1}), ester carbonyl band of oil extracted into acetonitrile; E (1631 cm^{-1}), HOH bending vibration.

Accordingly, the miscibility of various oils with acetonitrile was assessed by mixing the oil with acetonitrile in a 1:1 (w/v) ratio and measuring the height of the carbonyl band in the acetonitrile spectrum after phase separation was complete. The oils were determined to be slightly miscible (~0.5%) to an extent that varied somewhat with the overall degree of unsaturation, which could mean that moisture determination using the primary (water/acetonitrile) calibration could be affected by a dilution error that would depend on the type of oil analyzed. To investigate this possibility, five oils (olive, corn, safflower, peanut, and sunflower), all pre-dried over molecular sieves, were each spiked with three levels of moisture (approximately 100, 300, and 500 ppm) and analyzed in duplicate in accordance with the analytical protocol depicted in **Figure 5.4**. Regression (forced through the origin) of the means of the duplicates against the amount of moisture gravimetrically added to all the oils produced the following Z-reg relationship:

$$\text{Predicted } H_2O = 0.986 * \text{Added } H_2O$$

$$R^2 = 0.998 \quad SD = 8.1 \text{ ppm} \qquad [5.4]$$

Based on this experimental evidence, the use of calibrations based on simple water/acetonitrile standards was considered a suitable means by which to quantify moisture in edible oils.

5.4.3. Consideration of Interfering Constituents

Many minor constituents that may be present in oils can be extracted into acetonitrile and hence are potential sources of spectral interference in the quantitation of moisture by the method developed in the present study. Of particular concern are OH-containing molecules such as FFAs, hydroperoxides, and alcohols (glycerol, mono- and diglycerides). Indeed, as illustrated in **Figure 5.5**, which shows the overlaid differential spectra of acetonitrile spiked with glycerol, hexanol, *tert*–butyl hydroperoxide, oleic acid, and water, most of these constituents have bands that overlap significantly with one of the two OH stretching bands (3541 cm^{-1}) employed for quantitation of moisture. However, the higher-frequency OH stretching band (3629 cm^{-1}) is largely in the clear.

Although the HOH bending band (at 1631 cm^{-1}) does not suffer from interferences from OH-containing constituents, the use of this band for quantitation of moisture is not optimal because of the resulting ~2-fold reduction in sensitivity (cf. Eqs. [1]-[3]).

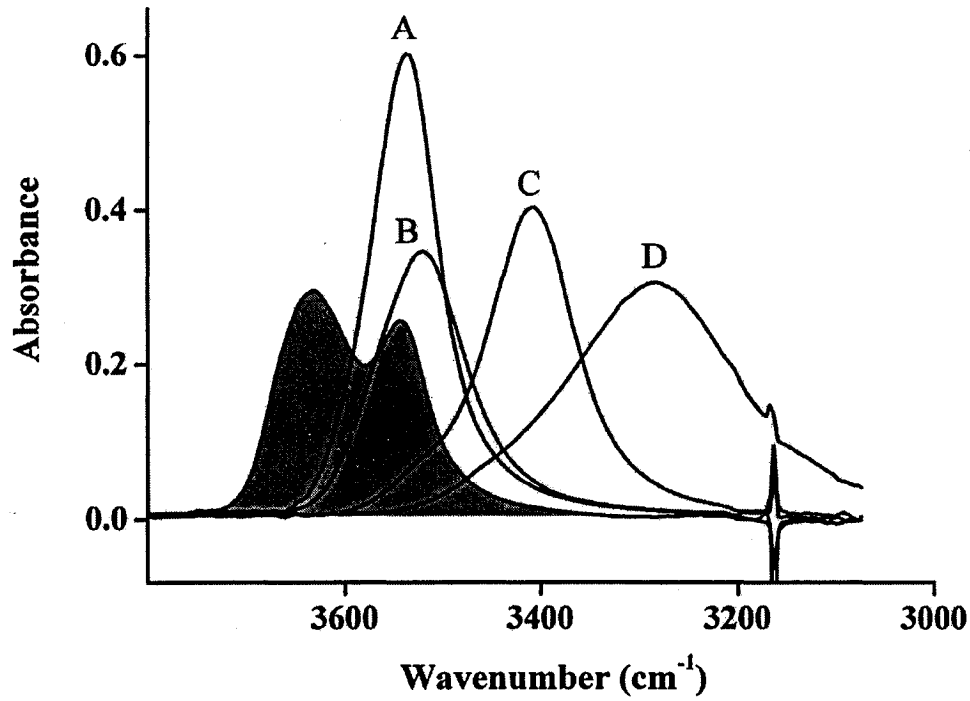


Figure 5.5. Differential spectra of acetonitrile spiked with hexanol (A), glycerol (B), *tert*-butyl hydroperoxide (C), oleic acid (D), and water (shaded). *Note*: The spectra are not on the same scale.

The effects that OH-containing constituents present in an oil would have on the accuracy of the method was evaluated quantitatively by analyzing 24 standards prepared gravimetrically by spiking corn oil with water (0-1000 ppm) and further spiking portions of each of these standards with (i) oleic acid (0.09-0.8% w/w), (ii) glycerol (0.05-0.1% w/w), or (iii) *tert*-butyl hydroperoxide (0.1-1% w/w). Comparison of the moisture predictions for the 18 standards in subsets (i), (ii), and (iii) with those for the 6 standards spiked only with water demonstrated that the predicted values obtained using the 3541

cm^{-1} band were high, roughly in proportion to the amount of "contaminant" added; the largest effect was observed for glycerol, as would be expected given the extensive band overlap illustrated in **Figure 5.5**, followed by the hydroperoxide and then the FFA. On the other hand, the predictions of moisture content based on either the 3629 or the 1631 cm^{-1} band showed no such effects. Regression of the predicted moisture contents for an additional subset, prepared by spiking four water/oil standards with randomized amounts of all three contaminants, against the "expected" values (calculated from the predictions for the standards by correction for the dilution due to spiking) yielded the following Z-reg equations for the 3629 and 1631 cm^{-1} bands, respectively:

$$\text{Predicted } H_2O_{3629\ cm^{-1}} = 1.054 * \text{"Expected" } H_2O$$

$$R^2 = 0.998 \qquad SD = 11.9 \text{ ppm} \qquad [5.5]$$

$$\text{Predicted } H_2O_{1631\ cm^{-1}} = 1.050 * \text{"Expected" } H_2O$$

$$R^2 = 0.997 \qquad SD = 18.3 \text{ ppm} \qquad [5.6]$$

These results confirmed that peak height measurements at 3629 or 1631 cm^{-1} allowed for the accurate quantitation of moisture in oils in the presence of OH-containing constituents at the levels commonly associated with edible oils.

5.4.4. Factors Affecting Analytical Accuracy

Owing to the ubiquitous nature of moisture in the environment, precautions were required to avoid moisture contamination of the sample handling system A simple, but effective means of minimizing moisture contamination was devised, as described in the Experimental section and depicted in **Figure 5 1**, without which reproducible calibrations over the range of 0-200 ppm could not be obtained. Above 200 ppm, the effects of environmental moisture were relatively minor; however, to cover all contingencies, this sample handling system was used routinely. Furthermore, in experiments conducted to determine the relative degree of analytical stability and reproducibility when samples were analyzed over a period of three days, deterioration of the analytical performance as time progressed was observed and eventually attributed to slow adsorption of water on

the walls of glass centrifuge tubes. This was confirmed by the finding that reproducible results were obtained in similar experiments when plastic centrifuge tubes fitted with septa were employed in place of capped glass tubes. The results of these experiments not only convey some of the precautions that must be taken to prevent loss or pickup of moisture when analyzing low levels of moisture in oil samples but also serve to illustrate the sensitivity of the FTIR method.

With proper precautions, very accurate data (on the order of ±10 ppm) can be obtained, based on standard addition experiments. For maximum sensitivity, the 3629 cm^{-1} OH stretch is the preferred measurement; however, the 1631 cm^{-1} band is useful for higher moisture levels (above 2000 ppm). Although not detailed in this paper, additional sensitivity (down to ~2 ppm) can be attained by increasing the pathlength (upper limit ~1000 μm) or using a 2:1 oil:acetonitrile ratio in the extraction step, or both. However, at such low moisture levels, the main limitation is not the sensitivity of the spectroscopic measurement per se, but rather the difficulty of ensuring against moisture pickup or loss, especially after extraction and during analysis.

5.5. ACKNOWLEDGEMENTS

The authors would like to thank Sultan Qaboos University, Oman, and the Natural Sciences and Engineering Research Council (NSERC) of Canada, for their financial support of this research. Thanks to Charles Chaney for suggestions related to this work and to Sophie Archelas for her technical assistance.

5.6. REFERENCES

1. O'Brien, R. D., *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing Company, Lancaster, PA, 1998.
2. Rossell, J. B. "Classical Analysis of Oils and Fats", in *Analysis of Oils and Fats*, R. J. Hamilton and J. B. Rossell, Eds., Elsevier Applied Science Publisher Ltd., London, England, 1986, Chap. 1, p. 52.
3. *Official Methods and Recommended Practices of the American Oil Chemists' Society* (AOCS, Champaign, IL, 1989), 4th edn.
4. Cedergren, A. and Luan, L. Potentiometric Determination of Water Using Spent Imidazole-Buffered Karl Fischer Reagents. *Anal. Chem.*, 70, 2174-2180 (1998).

5. Rosvall, M., Lundmark, L and Cedergren, A. Computer-Controlled, Coulometric Karl Fischer System for Continuous Titration of Water Based on Zero Current Potentiometry. *Anal. Chem.*, 70, 5332-5338 (1998).

6. Cedergren, A and Jonsson, S. Diaphragm-Free Cell for Trace Determination of Water Based on the Karl Fischer Reaction using Continuous Coulometric Titration. *Anal. Chem.*, 69, 3100-3108 (1997).

7. Cedergren, A. and Jonsson, S. Progress in Karl Fischer Coulometry using Diaphragm-Free Cells. *Anal. Chem.*, 73, 5611-5615 (2001).

8. Cedergren, A. Reaction Rates between Water and the Karl Fischer Reagent. *Talanta*, 25, 256-271 (1978).

9. Smith, B. *Infrared Spectral Interpretation: A Systematic Approach* (CRC Press, Boca Raton, FL, 1999), Chap. 1, p. 22.

10. Man, Y. B. C and Mirghani, M. E. S. Rapid Method for Determining Moisture content in Crude Palm Oil by Fourier Transform Infrared Spectroscopy. *J. Am. Oil Chem. Soc.*, 77, 631-637 (2000).

11. van de Voort, F. R., Sedman, J., Yaylayan, V., Saint Laurent, C. and Mucciardi, C. Quantitative Determination of Moisture in Lubricants by Fourier Transform Infrared Spectroscopy. *Appl. Spectrosc.*, 58, 193-198 (2004).

12. Critchfield, F. E. and Bishop, E. T. Water Determination by Reaction with 2, 2-Dimethoxypropane. *Anal. Chem.*, 33, 1034-1035 (1961).

13. Dix, K. D., Sakkinen, P. A., and Fritz, J. S. Gas Chromatographic Determination of Water Using 2, 2-Dimethoxypropane and a Solid Acid Catalyst. *Anal. Chem.*, 61, 1325-1327 (1989).

14. Le Narvor, A., Gentric, E. and Saumagne, P. Study of Binary Mixtures of Water with Several Proton Acceptors by Infrared Spectroscopy in the Region of OH Stretching Bands. *Can. J. Chem.*, 49, 1933-1939 (1971).

BRIDGE

The methods developed in Chapters 4 and 5 share a common analytical approach, namely the extraction of a constituent of interest from an edible oil into an oil immiscible solvent and its spectroscopic quantification. The development of these sensitive and accurate methods is only part of the solution in an industry that is looking to maximize efficiency and reduce costs through automation. Because the extraction based procedures use low viscosity solvents, they are amenable to being implemented on an automated system equipped with an auto-sampler. Chapter 6 describes the implementation of algorithms and procedures as well as validation of the methodology developed to effect the automated analysis of FFA and moisture in edible oils using such a system based on the methods described in Chapters 4 and 5.

CHAPTER 6

AUTOMATED FTIR ANALYSIS OF FREE FATTY ACIDS OR MOISTURE IN EDIBLE OILS

6.1. ABSTRACT

An FTIR spectrometer coupled to an auto-sampler and attendant methodologies for high-volume automated quantitative analysis of free fatty acids (FFA) and moisture in edible oils are described. Samples are prepared by adding 20 g of oil to a 50 ml screw-capped vial, to which is added either a methanol/NaHNCN solution or dry acetonitrile in a 1:1 (w/v) ratio for FFA or H_2O analysis, respectively. After capping with Mylar-lined septum caps, the vials are loaded into an auto-sampler tray, which is then agitated vigorously to extract the constituent of interest from the oil into the oil-immiscible solvent, and are then left to stand for ~10 min to allow for phase separation. The upper solvent layer in each vial is aspirated successively into the IR cell, with the Mylar seal allowing facile auto-sampler needle penetration into the vials. The spectra of the sample extraction solvents serve as spectral backgrounds as well being used to monitor cell path length and verify cell loading. FFA and H_2O analyses are carried out using 100 μm and 500 μm CaF_2 cells, respectively. For FFA analysis, quantification is achieved using the ν (COO^-) band at 1573 cm^{-1}, while moisture is determined using water absorption bands at 3541 cm^{-1} or 1631 cm^{-1}, depending on the moisture range of the samples. Calibration procedures and data are presented. The spectrometer and auto-sampler are controlled using proprietary UMPIRE Pro® (Universal Method Platform for Infra Red Evaluation) software, which provides a simple user interface and automates the spectral analysis; the output data can also be sent to a Laboratory Information Management System (LIMS). Validation and performance data obtained with this automated system demonstrate that it is capable of analyzing >60 samples/h, a rate commensurate with the throughput required by commercial contract or high-volume process control laboratories.

6.2. INTRODUCTION

Edible oils represent one of the primary constituents required for the formulation and manufacture of products by the food industry. Oils are extracted from a wide range of

raw materials and generally undergo relatively standardized extraction and refining processes and supplemental transformations such as fractionation, hydrogenation and/or interesterification prior to use. During processing, a variety of physico-chemical oil quality parameters are monitored, including the determination of free fatty acids (FFA), moisture (H_2O), peroxide value, iodine value, and saponification number, to name just a few[1]. Typically, QC laboratories have been limited to using standardized (AOCS[2] or IUPAC[3]) manual wet chemical methods to monitor oils in process, requiring a variety of analytical setups and multiple reagents as well as skilled personnel to carry them out, although these methods have been automated to some extent through the use of automatic titrators and flow injection systems.

The McGill IR Group has worked toward developing Fourier transform infrared (FTIR) spectroscopy as a rapid instrumental technique for the routine analysis of edible oils. Methodologies have been developed for the determination of FFA[4,5], saponification number[6], iodine value[6,7], *trans* and *cis* content[7,9], ansidine value[10], solid fat index[11], and peroxide value[12-14], amongst others. From the standpoint of implementation, most of these methods are semi-automated, with the software providing a simple user interface as well controlling the instrument, performing all calculations, and storing the results automatically. These FTIR methods provide improved sample turnaround and a reduction in reagent use relative to wet chemical methods. However, they do not provide the level of speed required by contract or centralized QC laboratories handling more than 100 samples/d, the key factor limiting sample throughput being the high viscosity of edible oils. On the other hand, in the case of new FTIR methods for the determination of FFA[15] and H_2O[16] in edible oils that have recently been developed in our laboratory, this limitation is obviated because the constituent of interest is extracted into a low-viscosity solvent prior to FTIR analysis. Indeed, these new FTIR methods have the potential for much higher sample throughputs than auto-titration systems, which presently provide the most rapid means of obtaining reliable FFA or moisture data. In addition to reducing the sample-flow constraints associated with high-viscosity oils, these extraction-based methods largely eliminate problematic matrix effects and provide much greater sensitivity than can generally be achieved by direct FTIR analysis of oils. This paper describes the instrumentation, analytical protocols, and software associated with an

automated FTIR/auto-sampler system designed to analyze for either FFA or H_2O in edible oils at rates of up to 100 samples per hour.

6.3. METHODOLOGY

Instrumentation. The analytical system used couples a Bomem WorkIR (Bomem, PQ, Canada) FTIR spectrometer with a Model 223 Gilson auto-sampler (Gilson, Inc., Middleton, WI) and a positive displacement micro-pump (Vissers Sales Corp., Aurora, Ont., Canada). The spectrometer is equipped with a DTGS detector and uses 100 and 500 μm CaF_2 transmission flow cells (International Crystal Laboratories, Garfield, NJ, USA) for FFA and H_2O analysis, respectively, with Luer-Lock cell connectors used to facilitate cell changes.

Software. The spectrometer, micro-pump and auto-sampler were all controlled by an IBM-compatible PC running proprietary Windows-based UMPIRE® Pro (Universal Method Platform for InfraRed Evaluation) software, developed by Thermal-Lube Inc. (Pointe-Claire, PQ, Canada). Built on the Microsoft.NET Framework, UMPIRE□ Pro software is designed to work with Windows XP or Windows 2000 operating systems. UMPIRE Pro is a self-contained platform that allows for the acquisition of spectra, analysis of spectral data, and cataloguing of results without operator intervention. For systems equipped with an autosampler and pump (such as that used in the present work), the analysis of all the samples loaded into the autosampler tray is fully automated. All parameters associated with the three fundamental tasks controlled by the software (spectral acquisition, data processing, and cataloguing of results) are predefined for each type of analysis (e.g., FFA determination, moisture determination) in an *analysis method*; additional customized analysis methods can be created using the method development tool provided in the software. The analysis method specifies all relevant acquisition parameters relating to the spectrometer and the auto-sampler, the component(s) to be quantified, and the *component method* associated with each component, where the latter specifies the spectral features that will be used for its quantification and the calibration (linear or polynomial) by which the results will be computed. All results are archived within the software and can be retrieved by referencing either the auto-sampler tray, analysis method, operator that acquired the samples, value of result, etc. These results can

be printed, exported to external software for further analysis or reporting, or re-processed by a different analysis method. Through the use of eXtensible Markup Language (XML) and the Microsoft.NET environment, UMPIRE Pro can relay information to a Laboratory Information Management System (LIMS).

Reagents. All solvents were of HPLC grade, obtained from Fisher Scientific (St. Louis, MO). Acetonitrile was dried over 4-8 mesh 4Å molecular sieves for a minimum of two weeks and dispensed using a re-pipette (Hirschmann-Laborgerate, Germany) protected by Drierite to prevent moisture ingress[16]. The reagent solution employed in the FFA analysis was prepared by dissolving NaHNCN in MeOH (2 g/L). This solution was allowed to stand for ~4 days or until the ν (C≡N) band at 2100 cm^{-1} completely disappeared before use[15]. Both extraction solvents (acetonitrile and methanol/NaHNCN) were kept in sealed bottles and maintained over molecular sieves.

Calibration Standards. For the FFA calibration, calibration standards were prepared by adding palmitic acid (0-1%) to canola oil which had been passed through activated silica gel column to remove any traces of FFA and other oxygenated compounds. Moisture calibration standards were prepared by gravimetric addition of distilled water (0-1000 ppm) to corn oil which had previously been kept over 10-20 mesh Drierite (calcium sulfate) for at least one week.

Analytical Protocol. Both the FFA and moisture analyses are based on extraction of the constituent of interest into an oil-immiscible solvent. For the analysis of FFA, the extraction uses methanol containing a weak base, hydrogen cyanamide (NaHNCN), the latter producing immediate conversion of acids into methanol-soluble salts without causing saponification of the oils, whereas for moisture analysis the sample is extracted with dry acetonitrile. The details associated with the procedures for these analyses have been described elsewhere[15,16]. **Figure 6.1** presents a generalized schematic diagram of the analytical protocol followed for both the calibration standards and the samples. For either FFA or moisture analyses, 20 g of oil was weighed into a tared 50-mL plastic vial to within ~±0.10 g. To the vial containing oil, 20 ml of the appropriate solvent was added using a calibrated pro-pipette, and the vial was capped. The vials were agitated on a vortex mixer for 30 s, and then loaded onto the autosampler tray. The thoroughly mixed

samples were then left to stand for 10-15 min to ensure separation of the oil and solvent layers. The automated FTIR spectral collection procedure was initiated with the collection of an open-beam spectrum followed by a single-beam solvent spectrum, the latter ratioed against the former to produce a reference solvent spectrum used to determine cell path length. The single-beam spectrum of the solvent layer in each sample vial was then recorded and ratioed against the single-beam solvent spectrum to obtain the spectrum of the constituent being analyzed for; the same single-beam solvent spectrum was used for a complete auto-sampler tray (56 samples). All spectra were collected by co-adding 32 scans at a resolution of 8 cm^{-1} and a gain of 1.0.

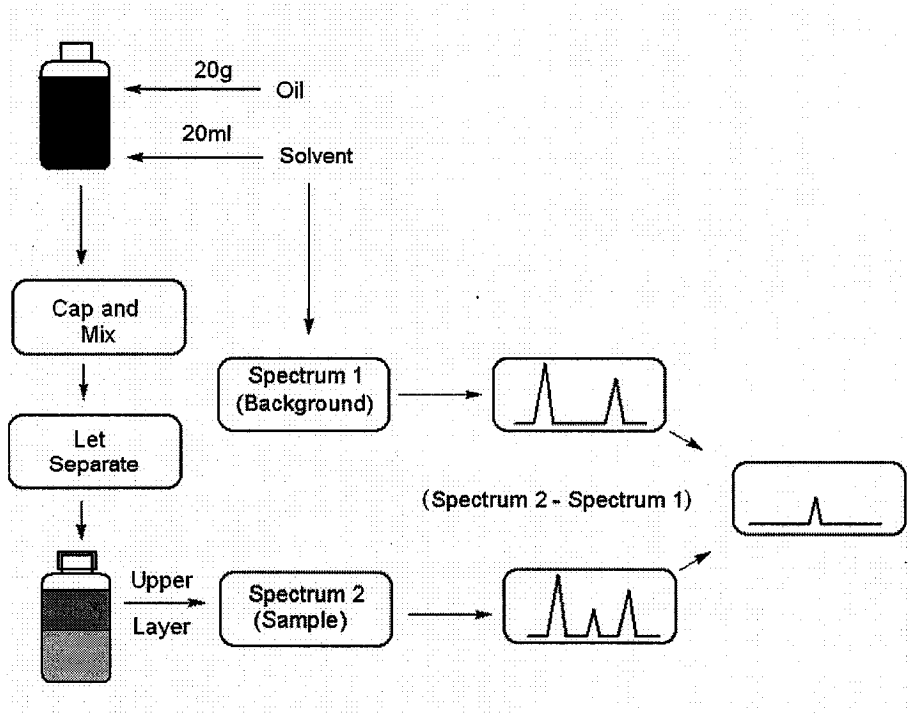


Figure 6.1. General analytical protocol for FFA and H_2O analysis of edible oils by FTIR spectroscopy.

For FFA analysis, the absorbance of the ν (COO^-) band at 1573 cm^{-1} relative to a baseline of 1820 cm^{-1} was measured and the FFA content predicted from a calibration equation obtained by regressing the corresponding absorbance values of the FFA calibration standards against their FFA content, expressed as % FFA (oleic acid). For moisture analysis, the calibration equations used were based on relating the amount of

moisture added to the moisture standards to the absorbance of the strong ν (O-H) band at 3629 cm^{-1} and/or the weaker ν (H-O-H) band at 1631 cm^{-1}, both measured relative to a single-point baseline at 2500 cm^{-1}. We have established empirically that measurement of the more sensitive 3629 cm^{-1} band is optimal up to a moisture level of 2000 ppm, beyond which the 1631 cm^{-1} band is preferred. The 2000 ppm limit corresponds to an absorbance at 3629 cm^{-1} of ~0.7, and the switchover from the 3629 cm^{-1} to the 1631 cm^{-1} calibration equation is automatically made by the software based on this absorbance criterion.

System Evaluation and Validation. Optimization of the analytical system in terms of minimizing sample carryover was initially evaluated by sequentially analyzing low (L) and high (H) FFA or moisture standards and calculating percent carryover as [(L2 – L1)/H1] *100. Sample carryover was further assessed by analyzing three replicates of six different oils of unknown FFA or H_2O content, these 18 samples being placed in the autosampler tray in random order. Subsequently, reproducibility and accuracy of the optimized automated FTIR method were assessed by standard addition of palmitic acid or water to olive, sunflower, and peanut oils, each validation sample being analyzed in duplicate, on different days, by the appropriate FTIR method and the results compared to the gravimetrically added amounts of these constituents.

6.4. RESULTS AND DISCUSSION

6.4.1. System Overview and Software

Figure 6.2 illustrates the overall system configuration. Because the FFA and moisture methods are designed to analyze low-viscosity solvent extracts rather than oils per se, a small micro-pump can be used to aspirate the sample into the IR cell. Low dead volume combined with 1/8" i.d. tubing minimizes sample volume (~15 ml required) and allows the elimination of solvent rinses between samples. The sample vials have conventional plastic septum caps but the silicone septum is replaced by a 20 mil Mylar liner. This Mylar liner provides the hermetic seal required for moisture analysis while being readily punched through by the auto-sampler needle **(Figure 6.3).**

Both the FTIR spectrometer and the auto-sampler are controlled by UMPIRE Pro, an upgrade of a proprietary software package designed as a general platform for developing and implementing FTIR methods. Details regarding the design of the software

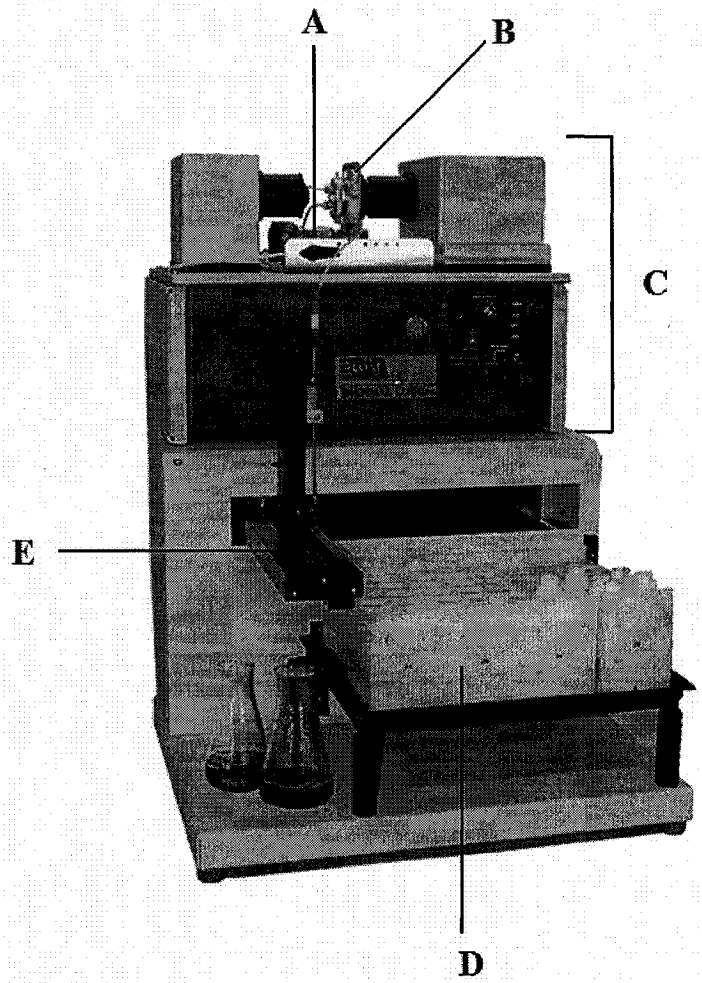


Figure 6.2. The FTIR/auto-sampler system used for the analysis of FFA or moisture. The system is composed of micro pump (A), cell holder (B), FTIR spectrometer (C), autosampler tray (D) and robotic arm (E).

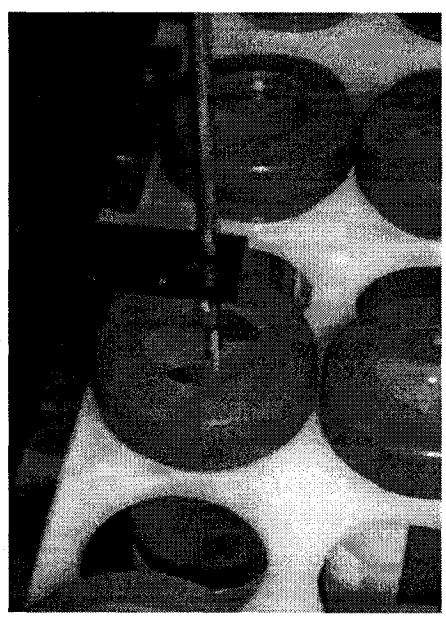


Figure 6.3. Auto-sampler needle punching through the Mylar liner.

are presented in the Methodology section, and some features of the user interface are illustrated in the panels of **Figure 6.4.** The user interface consists of a nested set of tab-based menus that allow the user to implement calibration and method development procedures. **Figure 6.4A** presents the "General" descriptive tab associated with the FFA method, and **Figure 6.4B** presents the Scanning/Sampling tab, from which the FTIR operating parameters and auto-sampler/pump parameters can be input. **Figure 6.4C** illustrates the typical tabs included under the "Components" submenu, showing the tabs that can be accessed from this submenu to define the peak height or area measurements to

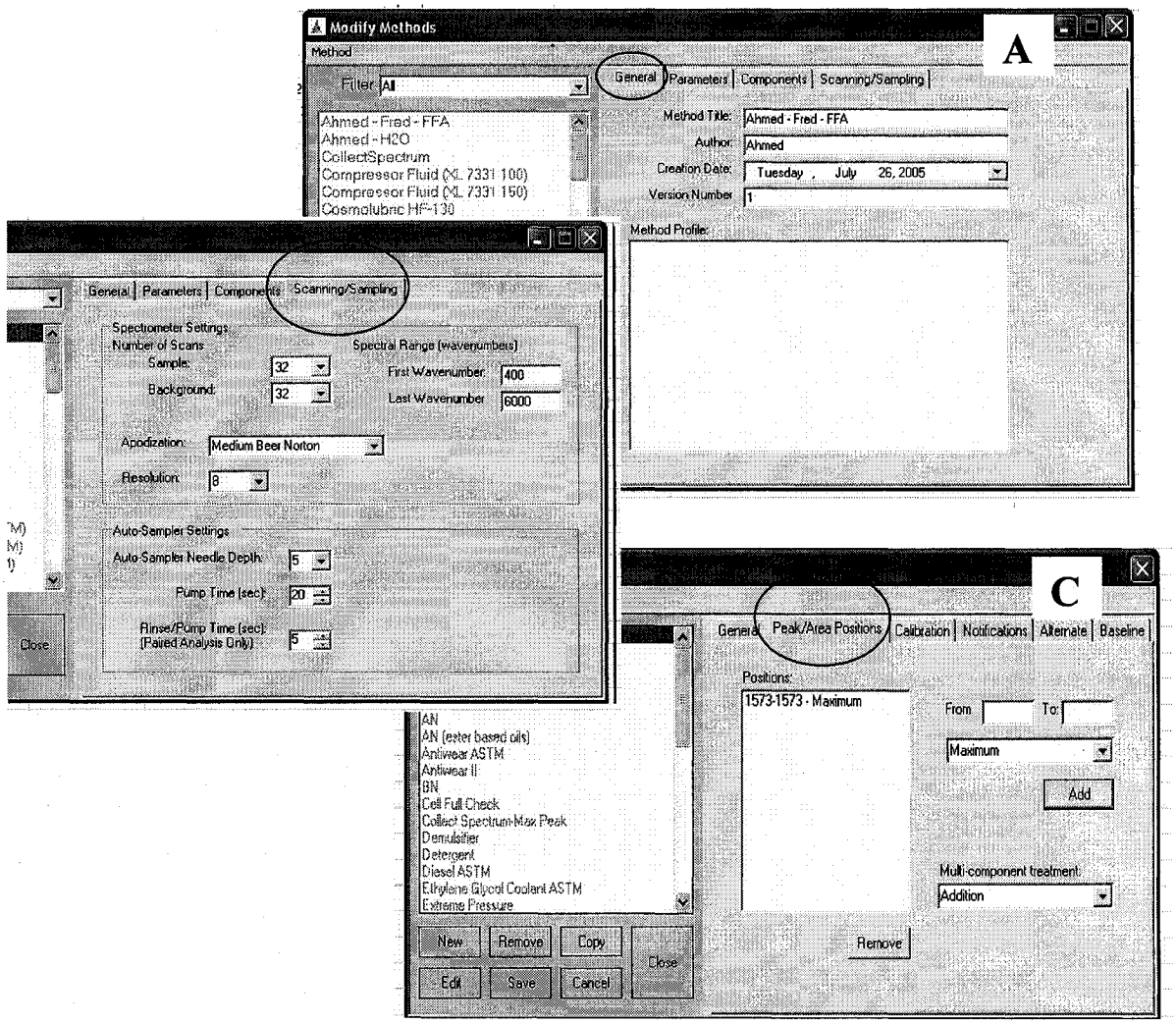


Figure 6.4. The user interface of UMPIRE® Pro program. A and B, the "General" and "Scanning/Sampling" tabs for the "Modify Methods" submenu, respectively; C, "Peak/Area Position" tab for the "Components" submenu.

be used for quantification and input calibration equations, warning messages for the operator, alternate equations to be used under defined conditions, and baseline correction procedures. This tab-based user interface makes it simple and intuitive to develop, assess, and modify methods as well as calibration procedures. In addition to these development and setup functions, spectra can be viewed, manipulated, and exported, and the analytical result(s) can be displayed, printed, trended and/or associated with a client, machine or operation as well as output to a Laboratory Information Management System (LIMS) for further processing or reporting.

For the present work, the autosampler was equipped with a 56-slot (7×8) tray. The first two slots of the auto-sampler tray were reserved for the solvent used for the analysis in question, the spectrum of which not only served as a background spectrum but also was employed to determine the cell path length. For the latter purpose, a path length calibration equation was developed by building three cells of different path lengths and relating their path lengths, as determined from the fringe count in the spectrum of the empty cell, to the height of an on-scale solvent band. Typical linear regression equations obtained for acetonitrile and methanol, respectively, were:

$$\text{Pathlength } (\mu\text{m}) = 673.51 * A_{2627/2580\ \text{cm}^{-1}} - 18.488$$

$$R^2 = 0.999 \qquad \text{SD} = 1.75 \qquad [6.1]$$

$$\text{Pathlength } (\mu\text{m}) = 261.99 * A_{2044/1952\ \text{cm}^{-1}} - 0.251$$

$$R^2 = 0.999 \qquad \text{SD} = 0.384 \qquad [6.2]$$

Using these equations, a cell path length is determined at the start of each run, of which an ongoing record is maintained to monitor the condition of the cell over time. This path length value is employed to normalize the spectral data subsequently collected for the samples in the tray to a constant path length (500 and 100 μm for H_2O and FFA, respectively). This procedure also allows one to rebuild cells without performing a recalibration of the method.

Once the auto-sampler tray is loaded with samples in capped vials, the whole tray is shaken vigorously to extract the constituent of interest from the oil into the solvent and then the samples are left to stand until phase separation occurs (~10 min). Automated analysis of the samples is then initiated. For each successive sample in the tray, the autosampler needle punches through the Mylar liner of the cap into the upper solvent layer, which is then aspirated into the IR cell and its spectrum recorded. A "cell-full" check is performed on the spectrum by measuring the height of a suitable solvent band in a spectral region in which the component of interest does not absorb (generally, the same band as used for the path length calculation); if this height is not within specifications, the analysis is paused, and the operator is notified to check for air bubbles in the cell or incomplete cell loading. When the "cell-full" check passes, the value of the parameter of interest is automatically calculated from the calibration equation defined for the method.

Figure 6.5 and **Figure 6.6** illustrate the differential spectra (solvent subtracted out) obtained for moisture and FFA standards, respectively, after extraction with the appropriate solvent. The calibration equations developed using these spectra were as follows:

$$H_2O \text{ (ppm)} = 3418.70 * A_{3629\ cm^{-1}} - 61.89$$

$$R^2 = 0.998 \qquad SD = 23.8 \qquad [6.3]$$

$$\text{FFA (\% w/w)} = 5.05 * A_{1573\ cm^{-1}} - 0.008$$

$$R^2 = 0.999 \qquad SD = 0.0018 \qquad [6.4]$$

These calibration equations were obtained using calibration standards prepared by spiking oils with the constituent of interest and extracting them with the appropriate solvent in accordance with the analytical protocol employed for sample analysis. However, since the spectral analysis is performed on the upper solvent layer containing the extracted constituent, the matrix effects associated with the oils are effectively eliminated, and hence the calibration procedure could be simplified substantially by spiking the constituents directly into their respective solvents.

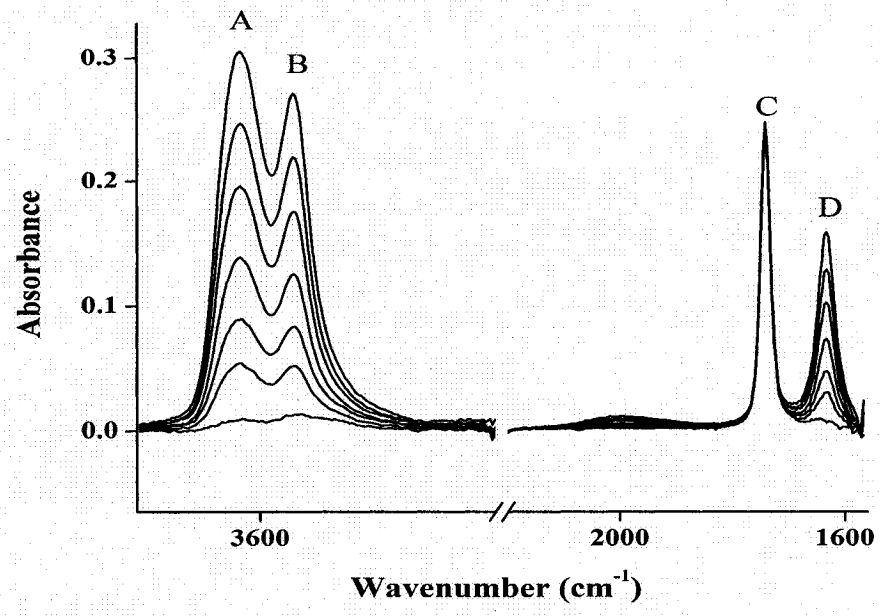


Figure 6.5. Differential spectra of canola oil standards spiked with water (0–2000 ppm) after extraction with acetonitrile. The major spectral features are the strong O-H stretching vibrations @ 3629 (A) and 3541 cm^{-1} (B), respectively, the ester carbonyl band due to traces of triacylglycerol being extracted into acetonitrile @ 1740 cm^{-1} (C), and the weaker HOH bending vibration of water @ 1631 cm^{-1} (D).

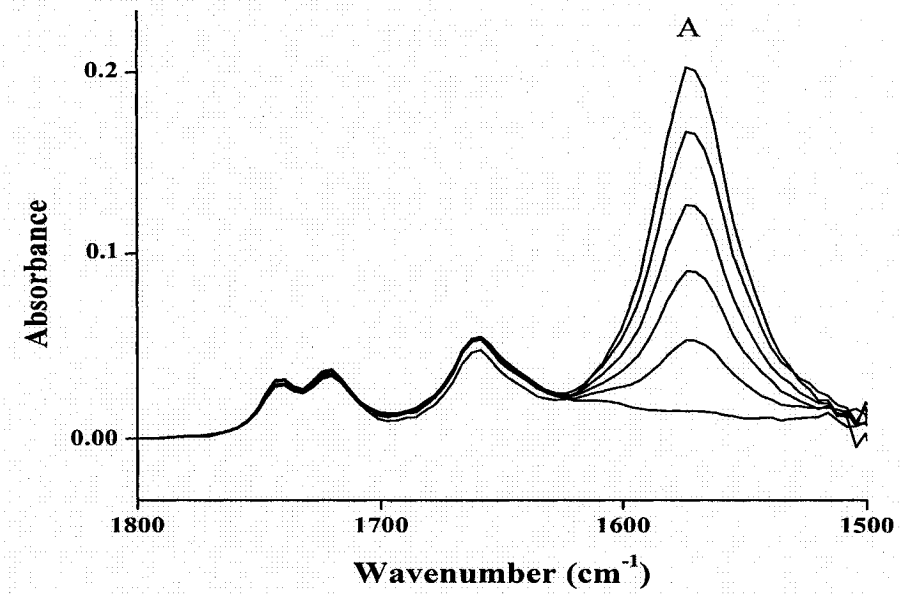


Figure 6.6. Differential spectra of canola oil standards spiked with palmitic acid (0.0–0.1% w/w) after extraction with methanol containing NaHNCN. The spectra illustrate the response of the FFA salts produced which absorb @ 1573 cm^{-1} (A).

6.4.2. System Evaluation and Validation

In optimizing the system parameters, the crucial variable is the pump time, the objective being to minimize sample turnaround time while avoiding sample carryover. The optimum pump time is largely a function of solvent viscosity and the wash volume required to flush out the previous sample. Preliminary trials for setting the pump time simply involved the analysis of high, intermediate and low FFA or moisture standards placed in the auto-sampler tray in random order. Based on this screening work, it was found that consistent results, without any carryover bias, were obtained using pump times on the order of 30 s (corresponding to a sample volume of ~15 ml). with negligible carryover (<0.5%). Subsequent to these trials, a series of three replicate sets of six oils (olive, sunflower, canola, sesame, grape seed, and safflower) of unknown FFA and moisture content were prepared for analysis. These 18 samples were loaded into the auto-sampler tray in random order for analysis by each method. **Table 6.1** presents the analytical data obtained for these runs. The excellent SD values confirm that sample carryover was negligible.

Table 6.1. FFA and Moisture Content of Various Edible Oils (mean of triplicate analyses)

Oil	FFA		Moisture Content	
	%	SD	ppm	SD
Canola	0.019	$2.9*10^{-3}$	39	1.9
Extra virgin olive	0.270	$10*10^{-3}$	850	12.3
Sunflower	0.024	$2.9*10^{-3}$	145	9.8
Safflower	0.024	$5.8*10^{-3}$	87	5.2
Sesame	1.170	$2.6*10^{-3}$	302	1.9
Grape seed	0.025	$10*10^{-3}$	168	12.0

Subsequently, a series of validation samples were prepared by gravimetrically spiking refined olive, corn, peanut, and sunflower oils with random, but known amounts of water (100–1000 ppm) and FFA (0.1–1%). These samples were analyzed in duplicate using the appropriate FTIR method for each constituent. The FTIR data are plotted against the amounts of moisture or FFA added to these oils in **Figure 6.7** and **Figure 6.8**,

respectively, and the corresponding zero-regression equations (forced through the origin) are presented in Eqs. [6.5] and [6.6].

$$\text{FTIR } H_2O = 1.007\ H_2O$$
$$R^2 = 0.997 \qquad \text{SD} = 14 \text{ ppm} \qquad [6.5]$$

$$\text{FTIR FFA} = 1.011\ \text{FFA}$$
$$R^2 = 0.999 \qquad \text{SD} = 0.007\ \%\ \text{FFA} \qquad [6.6]$$

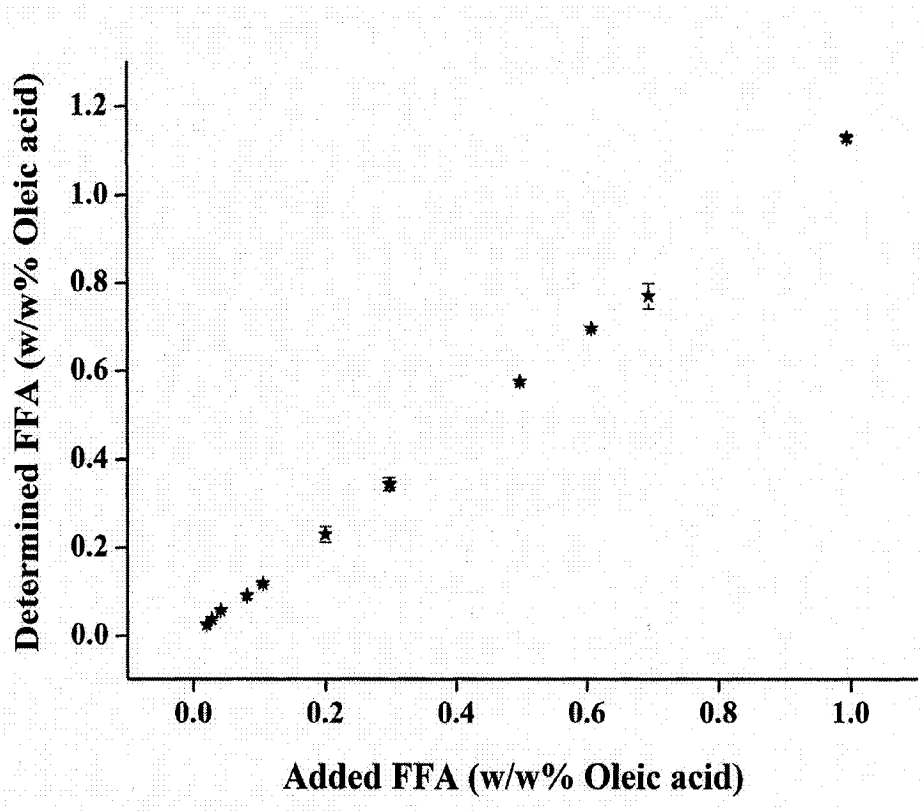


Figure 6.7. Plots for moisture content *vs.* amounts added for the validation run. Error bars represent mean ± SD of three replicates.

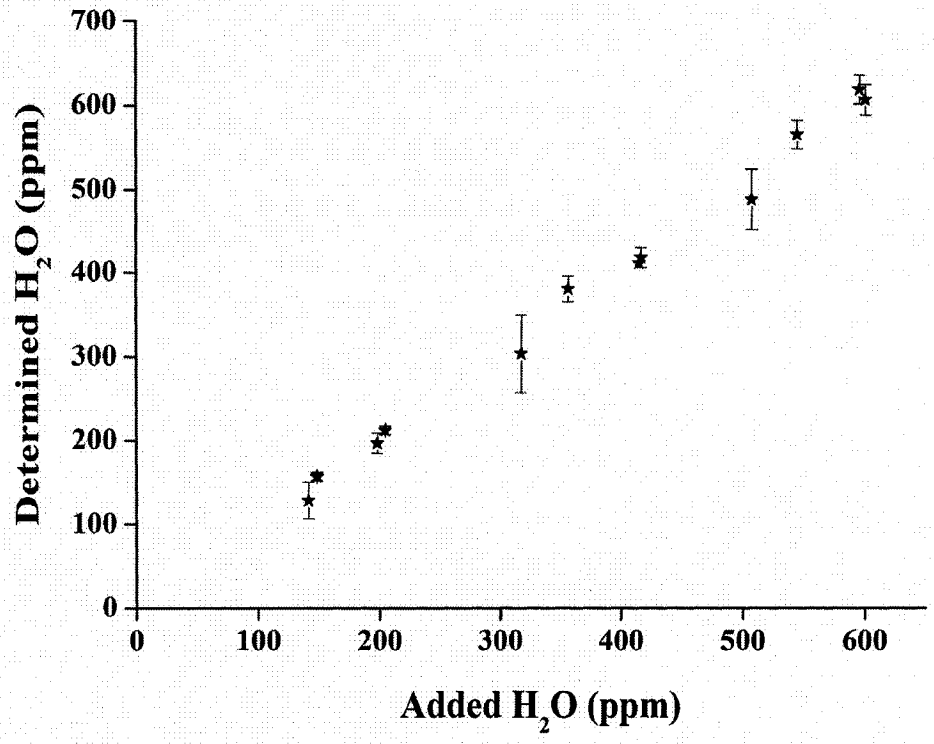


Figure 6.8. Plots for FFA *vs.* amounts added for the validation run. Error bars represent mean ± SD of three replicates.

6.5. CONCLUSION

This study provides evidence that the automated, high-speed analysis of edible oils for FFA and moisture using a FTIR spectrometer coupled to an auto-sampler is workable. Following sample preparation, which involves a simple in-vial extraction of the constituent in question into a low-viscosity solvent, and loading of samples onto an auto-sampler tray, the analysis is fully automated, with the FFA or moisture content results being output on-screen or to a LIMS. When optimized, throughputs of ~100 samples/h can be achieved, which meets the requirements of high-volume contract, process, or payment laboratories. It is also likely that the instrument configuration described in this paper can be adapted to other FTIR-based methods such as the determination of peroxide value or *trans* content.

6.6. ACKNOWLEDGMENT

The authors would like to thank Sultan Qaboos University, Oman, and Thermal-Lube Inc., Pointe-Claire, PQ, Canada for their financial support of this research. Thanks also go to Dr. R. Cocciardi for his technical assistance.

6.7. REFERENCES

1. O'Brien, R. D., *Fats and Oils: Formulating and Processing for Applications*, Technomic Publishing Company, Lancaster, PA, 1998.
2. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, IL, 1998.
3. IUPAC, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn., Blackwell Scientific, London, 1992.
4. Ismail, A. A., van de Voort, F. R. and Sedman, J. Rapid Quantitative Determination of Free Fatty Acids in Fats and Oils by FTIR Spectroscopy. *J. Am. Oil Chem. Soc.*, 70, 335-341 (1993).
5. Al-Alawi, A, van de Voort, F. R. and Sedman, J. New Method for the Quantitative Determination of Free Fatty Acids in Oil by FTIR Spectroscopy. *J. Am. Oil Chem. Soc.*, 81, 441-446 (2004).
6. Li, H., van de Voort, F. R., Sedman, J. and Ismail, A. A. 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).
7. van de Voort, F. R., Ismail, A. A. and Sedman, J. 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).
8. Li, Hui; van de Voort, F. R., Ismail, A. A., Sedman, J. and Cox, R. Trans Determination of Edible Oils by Fourier Transform Near-Infrared Spectroscopy. *J. Am. Oil Chem. Soc.*, 77, 1061-1067 (2000).
9. Sedman, J., van De Voort, F. R. and Ismail, A. A. 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).
10. Dubois, J., van de Voort, F. R., Sedman, J., Ismail, A. A. and Ramaswamy, H. R. Quantitative Fourier Transform Infrared Analysis for Anisidine Value and Aldehydes in Thermally Stressed Oils. *J. Am. Oil Chem. Soc.*, 73, 787-794(1996).
11. van de Voort, F. R., Memon, K. P., Sedman, J. and Ismail, A. A. Determination of Solid Fat Index by Fourier-Transform Infrared Spectroscopy. *J. Am. Oil Chem. Soc.*, 73, 411-416 (1996).

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

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

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

15. Al-Alawi, A., van de Voort, F. R. and Sedman, J. A New FTIR Method for the Analysis of Low Levels of FFA in Refined Edible Oils. *Spectrosc. Lett.* 38, 389-403 (2005).

16. Al-Alawi, A., van de Voort, F. R. and Sedman, J. A New FTIR Method for the Determination of Low Levels of Moisture in Edible Oils. *Appl. Spectrosc.*, (in press, 2005).

CHAPTER 7

GENERAL CONCLUSION

The methods documented in this thesis add a significant portion to the continuing work of FTIR methodology development in oil analysis. Each part of the work laid out in this thesis provides a solution to a common problem in its field or presents an improved means of carrying out edible oil analysis. The first method described in Chapter 3 provides a novel and yet workable approach to overcome matrix effects which for years hindered development of a general method for free fatty acids analysis of edible oils. The method uses a suspension of a weak base, potassium phthalimide (K-phthal) in 1-propanol (1-PrOH), to convert FFAs present in oils to their carboxylate salts without causing oil saponification. The matrix effects were resolved by splitting the diluted oil samples into two halves, with one half treated with the K-phthal reagent and the other half with 1-PrOH (blank reagent). The spectra of the two halves were then collected, and a differential spectrum obtained to ratio out the invariant spectral contributions from the oil sample. Quantification of FFA was based on direct measurement of the peak height of the free fatty acids salt band at 1570 cm^{-1}. Although this new method did not provide a substantial gain in terms of sensitivity ($\geq$ 0.2% FFA) over the standard and other titrimetric methods, it offers both specificity to free fatty acids and analysis independent of oil type, with differential spectroscopy being employed to circumvent matrix effects. Moreover, unlike the titrimetric methods, this FTIR method does not require reagent standardization, electrode conditioning or maintenance.

The subsequent FFA method described in Chapter 4, takes a different approach to the same analysis that makes it simpler to perform, more rapid and more sensitive without losing specificity. The method is extraction-based and the target constituent (FFA in the form of its salt) is extracted from oil samples using a polar oil-immiscible solvent (methanol) containing a weak base (sodium hydrogen cyanamide) which is soluble in the extracting solvent. Not only does this simplify the extraction procedure but it also significantly enhances sensitivity and obviates oil matrix effects as the oil is no longer present in the sample being analyzed spectroscopically. This method provides a simple and robust means by which to analyze for low levels of FFA with a high degree of

accuracy (± 0.0006%) with low solvent consumption (2 ml/analysis). Furthermore, with a simple adjustment of the reagent:oil ratio, the method is scalable for the analysis of semi-refined and crude oils containing up to 4.0% FFA. An additional benefit of this method is that its sensitivity opens the door to more accurate monitoring of the refining processes carried out on edible oils and the possible use of FFAs as an indicator of lipid oxidation.

In Chapter 5, the same principle of extraction and concentration was used to develop a rapid, practical, and accurate FTIR method for the determination of moisture content in edible oils. This was possible because of the high polarity of water, its strong mid IR absorption as well as the unique solvating characteristics of acetonitrile obviating the need to carry out any conversion reactions to produce more IR-active species. For the greatest sensitivity (0-200 ppm) the -OH stretching band (3629 cm^{-1}) is used, while for higher moisture levels (200-2000 ppm) the H-O-H bending band (1631 cm^{-1}) of water is suitable. One unique feature of the method is that the acetonitrile solvent does not need to be absolutely dry to obtain accurate results, as the method corrects for traces of moisture present via spectral ratioing against the acetonitrile used to extract the sample, greatly simplifying the methodology. It was also shown that alcohols, hydroperoxides, and FFAs, which may be extracted into acetonitrile, do not interfere with the analysis. This robust method has the potential to be a practical alternative to the Karl Fischer method, which has many variables associated with its analytical performance.

The work in Chapter 6 concerns the implementation of the FFA and moisture extraction-based methods on an FTIR spectrometer coupled to an auto-sampler to automate these analyses. The system developed effectively automates sample loading, spectral collection and calculation of results using pre-programmed algorithms, with output to screen or to a Laboratory Information Management System. It was tested, optimized and validated, and shown to be capable of sample throughputs of >60 samples/h with an accuracy very similar to that attained by using the manual procedures. This system is designed for high-volume contract, process or payment laboratories and lays the groundwork for extending its utility to other common edible oil analyses, such as peroxide value or *trans* content.

The development of the methods described in this thesis has been based on the design of novel approaches to problems that have hindered the full exploitation of the power of FTIR spectroscopy as a rapid and simple instrumental tool for edible oil analysis. This research has provided very accurate and specific measurements for two of the most important quality factors associated with edible oils. FFA and moisture methods have been developed that are superior in specificity and simplicity to their respective conventional titrimetric methods while providing comparable or better sensitivity and accuracy. Furthermore, the integration of an FTIR spectrometer with an auto-sampler, pump and appropriate software has made automated analysis of edible oils for FFA and moisture a realistic possibility. The adoption of such methods by industry can have a significant impact on the monitoring of oil processing operations and product quality control.