Application of a Handheld Portable Infrared Sensor to Monitor Oil Quality

A Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Masters of Science in the Graduate School of The Ohio State University

By

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2010

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ABSTRACT

Advances in infrared technology have made it a promising method for the food industry. The development of the Fourier Transform (FT) algorithm has shortened scan time, improved signal-to-noise ratio, and improved the accuracy of wavelength. Innovations in personal computing has made FT-infrared (FTIR) spectroscopy common place in quality control labs. Analysts routinely combine FTIR spectra with multivariate analysis to quantify components in their matrix.

Food scientists have combined FTIR with chemometrics to detect adulteration in their products. Both near- (NIR) and mid- (MIR) infrared have proven to be valuable resources for rapidly authenticating the quality of food. NIR has been applied to products such as juice, honey and milk, and for detecting acrylamide in potato chips. MIR has been successful in authenticating edible oils, juices, honeys, and for ensuring products are correctly identified as organic. As food safety continues to be a top priority for consumers, there is no doubt that improvements will continue to be made on spectrometers and in chemometric methods in order to stay one step ahead of adulterators.

One such improvement has been the development of handheld portable infrared sensors. These units are used for chemical identification in the homeland security, public safety, pharmaceutical, industrial, and medical markets. They bring the spectral
resolution of benchtop instruments to field applications in rugged, battery operated units weighing less than four pounds.

A novel application of a handheld FTIR is for monitoring oxidation in edible oils. Methods currently used for oil quality testing are subjective, time consuming, and use hazardous solvents which then need to be disposed of. The aim of this research was to evaluate the capabilities of a handheld FTIR combined with multivariate analysis to characterize frying oils and to monitor chemical processes occurring during lipid oxidation as well as determining fatty acid composition.

Commercial frying oils (corn, peanut, sunflower, safflower, cottonseed and canola) were heated to 65°C in an oven for thirty days to accelerate oxidation. Reference methods included fatty acid composition (IUPAC 2301, 2302), peroxide values (PV (AOCS Cd 8-53) and free fatty acid (FFA, Shipe 1979). Aliquots were drawn at five day intervals and analyzed by benchtop and handheld mid-infrared devices and reference methods. Spectral analysis (Soft Independent Model of Class Analogy (SIMCA) and Partial Least Squares Regression (PLSR)) was carried out by pattern recognition software.

Mid-infrared spectral regions ~3000-2825 cm\(^{-1}\) (C-H stretching bands) and 1740 cm\(^{-1}\) (C=O stretching of esters) were important for classification. All six oil samples formed distinct and well-separated clusters in the SIMCA plot due to difference in their chemical composition. It was found that spectral variability could be minimized by controlling oil temperature (65°C) during data acquisition. PLSR showed good correlation coefficients (Rval) between FFA and PV on the infrared devices.
Handheld IR instruments combined with multivariate analysis showed promise for determination of oil quality parameters with similar performance as the benchtop units. Its portability and ease-of-use make the handheld IR a great alternative to traditional testing methods.
ACKNOWLEDGEMENTS

I would like to thank Dr. Luis Rodriguez-Saona for his guidance in this project. Without his patience, this project would not have been possible.

I would also like to thank my amazing lab group for their encouragement and support and for their never ending supply of candy!

Finally, I would like to thank my family and friends for putting up with my moments of insanity during my time as a graduate student.
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Chapter 1

LITERATURE REVIEW

1.1 LIPIDS

Lipids are among the oldest classes of chemical compounds used by humans (Dupuy and others 1996). Fats and edible oils are concentrated sources of energy that contribute to the palatability of food by improving the texture and providing a more desirable flavor (Nawar 1996). Recently there has been a shift in the source of fats and oils consumed in the United States due to consumers being more aware of the role foods play in their health. Vegetable oils are increasingly important because of their high content of mono- and poly-unsaturated fatty acids. These are known to have several health benefits including reducing the risk of heart disease when compared to saturated fats (Muik and others 2005; Jakobsen, O'Reilly, Heitman 2009).

Annual consumption of edible oils in the US has reached 22.5 billion pounds. Of this, approximately 25% is used for frying foods (US Census Bureau 2009). There are several types of vegetable oils that are commercially used as a frying medium including canola, corn, peanut, safflower, cottonseed, soybean, and sunflower oil (Matthaus 2007). Canola is processed from a variety of the rapeseed plant that is low in erucic acid and is recognized for being low in saturated fat (6%). Corn oil is produced as a by product of cornstarch production and is known for having high levels of polyunsaturated fatty acids (55%). Peanut oil is used primarily for deep frying and as cooking oil but is relatively
expensive. Safflower oil’s high linoleic content (75-80%) is the highest of available oils. Its use is limited due to short supply and lack of flavor stability (Lawson 1995).

The United States Food and Drug Administration (FDA) has no specific regulations in place to control oils and fats or their use. There are no standards of identity but the FDA enforces accurate labeling of oils and fats under a general provision of the Federal Food Drug and Cosmetic Act. The American Oil Chemist’s Society (AOCS) is the key technical and methods development and approval organization worldwide for fats and oils (Lawson 1995).

Vegetable oils are extracted from seed fruits or nuts through solvent extraction. Crude vegetable oils have distinct undesirable flavors but these oils are processed to attain the desired neutral flavor (Lawson 1995). Even after processing, edible oils still contain minor components that lower the quality of the oil. These components include: free fatty acids, phospholipids, carbohydrates, proteins, water, transition metals, and products of lipid oxidation. In processed soybean oil, for example, these minor fractions make up 3% of the oil. Of this, phospholipids account for 1.5-2.5%. At concentrations above 0.1%, phospholipids cause the development of off flavors and darkening of the oil as a result of maillard browning. Transition metals are found in edible oils because these metals originate in the soils in which the oil-bearing plant was grown or from equipment used during processing and storage. These metals act as prooxidants even at levels as low as 0.1 PPM (Mistry and Min 1992).

The term lipid refers to a wide range of compounds that are soluble in organic solvents but not in water. In contrast, the terms fat and oil often refer to bulk products of commerce that have already been extracted from animal products or oil seeds and other
plants grown for their lipid content (Nielsen 2003). Oils are triglycerides of various fatty acids esterified to glycerol, differing in length and degrees of unsaturation of the fatty acid chains (Guillen and Cabo 1998; Nawar 1996)(Figure 1).

![Structure of a triglyceride](image)

**Figure 1:** Structure of a triglyceride

*Source:* WKU BioWeb, 2009

Autoxidation, a process pioneered by Farmer et al., Bateman and Morris, is the major deteriorative reaction affecting edible oils and is of primary concern to processors and consumers from a quality standpoint (Farmer and others 1941; Bateman and Morris 1953). Autoxidation results in rancidity- the unpalatable odor and flavor of edible oils which have undergone oxidative or hydrolytic degradation. Oxidative rancidity involves oxygen attack of glycerides and occurs in all unsaturated fats (Mistry and Min 1992). Free fatty acids are more prone to oxidation than triglycerides so their presence in fats and oils increases the possibility of rancidity (Guillen and Cabo 1997).

Lipid oxidation is a free radical mechanism that occurs in three phases: initiation, propagation, and termination. Initiation occurs at the same time as the first free radical is formed from the loss of a hydrogen. Propagation is a chain reaction of free radical formation. In the presence of oxygen, the first free radical turns into peroxide (a free
radical) which attacks another fat molecule. This molecule, after losing a hydrogen atom, generates a hydroperoxide next to another free radical. The most weakly bound hydrogen is the one that is removed (Moros and others 2009). Propagation is the slowest rate determining reaction during the course of autoxidation (Mistry and Min 1992). In the initial stages, the rate of formation of hydroperoxides exceeds their rate of decomposition. In later stages, it is the reverse. Termination is the point of product degradation (Lawson 1995; Muik and others 2005). At this point, there are noticeable changes in smell and color as a consequence of the formation of secondary oxidation products formed from hydroperoxide decomposition. Hydroperoxides start decomposing into various products as soon as they are formed (Mistry and Min 1992). The autoxidation reaction can be summed up as:

Initiation: RH + O₂ → R. + .OH  
Propagation: R' + O₂ → ROO'  
ROO' + RH → ROOH + R'  
Termination: R' + R' → RR  
R' + ROO' → ROOR  
ROO' + ROO' → ROOR +O₂

Where RH is any unsaturated fatty acid in which the hydrogen is susceptible because of being on a carbon atom adjacent to a double bond and R' is a free radical formed by removing a hydrogen (Gray 1978).

As unsaturation in the fatty acid tail of triglycerides increases, the rate of oxidation increases (Mistry and Min 1992). For instance, stearic, oleic, linoleic and linolenic acid all have the same 18 carbon backbone, but they differ in the number of points of unsaturation (Figure 2). Stearic acid is a saturated fatty acid with no double
bonds. Linolenic is the least saturated, with three double bonds (Nawar 1996). The relative rates of oxidation of stearic: oleic: linoleic: linolenic acid is 1: 100: 1200: 2500 (Mistry and Min 1992). As shown in table 1, these long chain fatty acids are found in virtually all types of edible oils.

Figure 2: Structure of stearic, oleic, linoleic and linolenic acid


Considerable care is exercised during manufacturing, storage, and use to slow down the oxidation process as much as possible (Lawson 1995). Where as other deteriorative reactions such as microbial or enzyme attack can be largely controlled by lowering the temperature, careful packaging, and sterilization, this does not prevent lipid oxidation since autoxidation is a chemical reaction with a low activation energy (4-5 kcal/mole) (Gray 1978; Allen and Hamilton 1994).
Table 1: Fatty acid composition of various edible oils

Percent by weight of total fatty acids

<table>
<thead>
<tr>
<th>Oil type</th>
<th>Saturated</th>
<th>Mono unsaturated</th>
<th>Poly unsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myristic acid</td>
<td>Palmitic acid</td>
<td>Stearic acid</td>
</tr>
<tr>
<td></td>
<td>C14:0</td>
<td>C16:0</td>
<td>C18:0</td>
</tr>
<tr>
<td>Canola Oil</td>
<td>-</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Corn Oil (Maize Oil)</td>
<td>-</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Cottonseed Oil</td>
<td>1</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>-</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Safflower Oil</td>
<td>-</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>-</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Sunflower Oil</td>
<td>-</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

Modified from: http://www.scientificpsychic.com/fitness/fattyacids1.html

Other preventative measures are necessary to slow the rate of autoxidation. In some instances, antioxidants are added to edible oils in order to inhibit the process. Antioxidants are substances which delay the onset or slow the rate of oxidation of oils, impeding the formation of oxidation bi-products. Their primary disadvantage is that they merely slow oxidative rancidity and not prevent it. Some hydroperoxides will have to initially form in order for the antioxidants to react. Antioxidants are unable to prevent rancidity if the peroxides are present in excess, such as in the later stages of oxidation (Mistry and Min 1992).
While negative qualities of food flavor are associated more closely with lipids than with proteins or carbohydrates, lipids are also responsible for good flavors and are used for deep frying (American Chemical Society Meeting and others 1994). Unfortunately, lipids from edible oils and fats suffer from thermal degradation when they are subjected to high temperatures for a long time, such as during frying. It is important to monitor frying oils during the heating process because the rate of oxidation increases with an increase in temperature, exposure to oxygen in the air, the presence of light, and contact with prooxidants (Moros and others 2009; Lawson 1995). Fats used for deep fat frying undergo chemical changes during use including color formation, oxidation, polymerization (reaction of fat with itself, forming larger fat molecules; results in foaming) and hydrolysis. Odor and off-flavor development are also significant because these are defects noticed by the consumer (Lawson 1995).

Lipid oxidation at high temperatures is complex because thermolytic and oxidative reactions occur simultaneously. Thermal oxidation produces various physical and chemical changes in the oil including formation of volatile compounds; an increase in viscosity, polarity, and fatty acids; formation of dark color; decrease in iodine value and surface tension; change in refractive index and a tendency of the oil to foam. Decomposition of the frying oil occurs due to thermal oxidation, isomerization, hydrolysis, pyrolysis, and polymerization of the triglycerides. Since oils are glyceride mixtures of various fatty acids, their decomposition products reflect the break down of their constituent fatty acids (Mistry and Min 1992).
1.2 TRADITIONAL METHODS FOR MONITORING OIL QUALITY

1.2.1 Wet Chemistry

Taste assessment is in the long run the most reliable method to measure oil quality because it measures what the customer actually perceives. Unfortunately, it is difficult to ascribe numerical data to organoleptic assessment in order to make comparisons with results obtained on a different date or in a different location. It can also be expensive and cumbersome to carry out (Allen and Hamilton 1994). It is for these reasons that many people look to physical and chemical methods for rancidity measurements. Numerous analytical protocols have been developed for measuring early stages of lipid oxidation including spectrophotometric, iodometric, and colorimetric methods (Wrolstad 2005). These methods measure the concentration of primary or secondary oxidation products (Moros and others 2009).

Peroxide value (PV) is one of the most widely used methods to determine the rate at which oxidation progresses (Guillen and Cabo 2002). It is a measure of the state of oxidation of an oil or fat and is expressed as milliequivalents (mEq) active oxygen per kilogram oil. PV is traditionally determined using wet chemistry methods (Guillen and Cabo 1997; Lawson 1995). The primary product of lipid oxidation is hydroperoxides, which is generally referred to as peroxides. Peroxide value is influenced by constituent fatty acids and the length or type of storage (Lawson 1995).

Being able to monitor and quantify the development of peroxides by objective means over time is important for food scientists who want to characterize the quality of oil. Even though peroxides themselves are not directly related to the actual sensory
quality of the product tested, their presence indicates the potential for later formation of sensoraly objectionable compounds (Wrolstad 2005). Hydroperoxides formed by autoxidation are very unstable and break down into a wide variety of volatile and nonvolatile flavor compounds. These secondary oxidation products include aldehydes, alkenes, alkanes, and alcohols. Aldehydes are the most significant and have been described as green, painty, metallic, beany or rancid and are often responsible for the undesirable flavors in oils and oil-containing foods (American Chemical Society Meeting and others 1994). Refined oils may be found to have PVs of up to ten before off flavors are encountered (Allen and Hamilton 1994).

Peroxides can be measured by titrimetric methods based on their potential to oxidize iodide ($I^-$) to iodine ($I_2$). This is the official AOCS iodometric method for PV determination (Cd 8-53) and it is applicable to all normal fats and oils. Iodine is liberated by hydroperoxides in oil in the presence of excess iodine. The amount of iodine present is determined by titration with a standardized sodium thiosulfate solution using a starch indicator, reflecting how much peroxide is present in the oil or lipid extract (Wrolstad 2005). The reaction can be written as:

$$ROOH + 2I^- \rightarrow ROH + I_2 + H_2O$$
$$I_2 + 2Na_2S_2O_3 \rightarrow (starch) Na_2S_4O_6 + 2NaI$$

There are a few sources of error in this method: absorption of iodine at unsaturated bonds of the fatty acid, and the liberation of iodine from KI by oxygen present in the test sample. The latter is referred to as the oxygen error and leads to false high results (Wrolstad 2005). Further, the iodometric titration method is highly empirical and any changes in procedure such as altering time and temperature may cause variation in results.
As a result, its accuracy is questionable and the results vary with the specific procedure employed. Low PVs cannot be adequately measured by official methods due to uncertainty with the iodometric titration end point. An additional drawback is that a large sample size (5 grams) is needed to carry out the titration (Wrolstad 2005).

Determining free fatty acid (FFA) levels in edible oils is a standard characterization technique in the food industry (Guillen and Cabo 1997). Any fatty acid not linked to a glycerol or some other molecule in an oil or fat is referred to as a free fatty acid (Lawson 1995). They are chemically less stable than triglycerides and therefore are more likely to oxidize and cause rancidity (Al-Alawi, van de Voort, Sedman 2004). Most unrefined oils contain relatively high levels of FFAs. Processing methods have been developed to reduce, but not eliminate, the amount of free fatty acids present in refined oils (Guillen and Cabo 1997). Refined oils and fats that are ready for use in foods usually have a free fatty acid level of less than 0.05%. Hydrolysis is the reaction of water with oil, producing free fatty acids (Lawson 1995). This reaction can be written as:

\[
\text{Triglyceride + water} \rightarrow (\text{w/ heat}) \text{glycerol + 3 FFA}
\]

Hydrolysis is accelerated by high temperatures and pressure, plus an excess of water. These conditions are common during the production of fried foods where fat is 350°F and the raw materials, such as potatoes, are high in moisture (Lawson 1995).

The standard method for FFA analysis is based on the titration of oil dissolved in alcohol with a strong base to a phenolphthalein end point. This method is tedious and
requires substantial amounts of solvent. Additional methods have been developed to measure FFA levels in edible oils (Al-Alawi, van de Voort, Sedman 2004). One such procedure was modified from a copper soap method that was developed as a way to determine FFA in milk. Free fatty acids are converted to copper soaps, extracted, and the copper reacted with a color reagent. The color reagent is measured on a UV-Vis spectrometer. A standard curve is constructed from solutions of known concentrations of palmitic acid and the concentration of FFA is extrapolated from the curve. The sensitivity of a colorimetric measurement is greater than titrimetric measurements and only a small sample (0.5mL) is needed (Shipe, Senyk, Fountain 1979). While this method produces accurate results, it is still a lengthy process and requires a large amount of hazardous chemicals.

### 1.2.2 Gas Chromatography

Gas chromatography (GC) is commonly used to separate and identify the products of lipid oxidation. The basis for GC separation is the distribution of an analyte between two phases. One of these phases is a stationary phase found inside a GC column and the other is a mobile gas phase which is essentially inert. Once the sample enters into contact with the mobile phase as it is forced through the column, the components of the sample interact to a varying extent with the stationary phase, resulting in differential migration rates through the column (Allen and Hamilton 1994).

To briefly describe the procedure, a sample is injected into the gas phase (the mobile phase) where it is volatilized and is carried onto the stationary phase which is found inside of a column installed in a temperature controlled oven. There are unlimited numbers of columns to choose from with varying stationary phase materials depending
on the type of material being analyzed. Components are eluted at different rates, depending on their affinity for the stationary phase, and emerge from the end of the column exhibiting peaks of concentration, ideally with a Gaussian distribution. These peaks are detected by a detector which converts the concentration of the component in the gas phase into an electrical signal, which is amplified and passed to a continuous recorder and to an integrator, so that the progress of the separation can be monitored and quantified (Figure 3). Changes to the mobile phase, stationary phase, length and diameter of the column, and temperature ramps can improve the resolution of peaks (Chrisie 2009).

![Typical gas chromatograph layout](Image)

**Figure 3:** Typical gas chromatograph layout


The flame ionization detector (FID) is the most common GC detector. It can be used with almost all organic compounds, has high sensitivity and stability, a low dead volume, a fast response time and the response is linear over an extremely wide range (Chrisie 2009; Pare and Belanger 1997). This detector is simple to construct and operate,
and it is highly reliable in prolonged use. These advantages make the FID a perfect choice for GC analysis of oils (Chrisie 2009).

The most common GC method for analysis of the fatty acid composition of edible oils is the conversion of fatty acids by methanol into fatty acid methyl esters (FAME) in order to improve their volatility, thereby ensuring better GC peak shape. FAME are conventionally synthesized from a lipid sample in a sequence of steps consisting of drying, hydrolysis, extraction, purification, and transesterification of the fatty acids. The final step is analysis using gas chromatography. This method is cumbersome, time consuming, and relatively expensive (Indarti and others 2005).

An alternative method combining extraction and transesterification into one step has been developed. It is simpler, faster, and uses considerably less organic solvents. This method uses a methanolic-H$_2$SO$_4$ reagent system which is added to the lipid sample and boiled. The addition of water and hexane extracts the FAME into the hexane layer for analysis (Rodriguez-Saona, Barrett, Selivonchick 1995). A capillary column with a polar stationary phase is traditionally used because it allows for complete separation of the FAME peaks (Eder 1995). While the direct FAME synthesis is an improvement compared to the traditional method, the entire process takes approximately 3 hours and still requires highly trained technicians.

1.2.3 Other analytical methods

Other less common analytical methods have been developed to measure the extent of oil oxidation. UV absorbance, measured at 233 and 268nm is employed to monitor the formation of conjugated dienes and trienes of polyunsaturated fatty acids. In order for conjugated dienes to form, the oil must contain unsaturated fatty acids with at least two
double bonds (e.g. linoleic acid). At least three double bonds (e.g. linolenic acid) are needed for conjugated trienes to form. Increasing absorption values are an indication that oxidation is proceeding. This physical method is performed on oils directly and is non-destructive (Wrolstad 2005).

Some methods for measuring lipid oxidation are based on determining the concentration of secondary oxidation products. These methods give information about the concentration of the corresponding compound or group of compounds. For instance, the carbonyl value measures the total content of aldehydes and ketones. This method has been criticized for giving erroneous results due to the degradation of hydroperoxides into carbonyl derivatives. The content of aldehydes in headspace or in oil can also be measured. Acid Value (AV) determines the concentration of alpha and beta alkenals. AV represents the content of secondary oxidation products and all of the compounds able to react with a p-anisidine reagent. Some authors have indicated that acid values are comparable only within each oil type because initial AV varies among oil sources (Guillen and Cabo 2002).

The thiobarbituric acid method (TBA) is a colorimetric reaction that measures the production of malonaldehyde. There has been extensive criticism of this method, including thiobarbituric acid reacting with compounds other than those found in the oxidizing system to produce a red pigment, giving false results. There is also poor correlation between TBA values and other indicators of oxidation (Gray 1978).
1.3 SPECTROSCOPY

Spectroscopy explores the production, measurement, and interpretation of spectra arising from the interaction of electromagnetic radiation with matter. The numerous methods available differ with respect to the subject analyzed, the type of radiation-matter interaction to be monitored (absorption, emission, or diffraction) and the region of the electromagnetic spectrum used in analysis (Nielsen 2003).

The objective of quantitative absorption spectroscopy is to determine the concentration of an analyte in a given sample solution. The determination is based on the measurement of the amount of light absorbed from a reference beam as it passes through the sample solution. The solution to be analyzed is contained in an absorption cell placed in the path of radiation of a selected wavelength. The relative amount of light passing through the sample is then used to estimate the analyte concentration (Penner 2003). Absorbance is directly proportional to the concentration of the absorbing species in the solution. The relationship is known as Beer’s law (Equation 1).

\[ A = \varepsilon bc \]

- \( A \) = absorbance
- \( \varepsilon \) = molar absorptivity
- \( b \) = pathlength (cm)
- \( c \) = molar concentration

**Equation 1:** Beer's law

The sample holding cell should be composed of a material that does not absorb radiation in the spectral region being used. For the UV region, quartz cells are appropriate, while silicate glass or plastic is acceptable for the visible region. It is important to choose a
wavelength at which the analyte demonstrates maximum absorbance and where the absorbance does not change rapidly with changes in wavelength. This typically corresponds to the apex of the highest absorbing peak (Penner 2003).

A basic spectrophotometer is composed of five essential components: the light source, the monochromator, the sample/reference holder, the radiation detector, and a readout device. The most common radiation source for visible spectrophotometers is a tungsten filament lamp and a deuterium electrical-discharge lamp for ultra-violet spectrophotometers. The monochromator isolates the specific group of wavelengths to be used in the spectroscopic assay (Penner 2003).

1.3.1 UV-Visible Spectroscopy

Ultra-Violet-Visible (UV-Vis) spectroscopy is a common analytical tool for studying oil quality. It is used for measuring FFA content and quantitating values of conjugated dienes and trienes. The analytical signal for which the assay is based is either the emission or absorption of radiation in the UV-Vis range of the electromagnetic spectrum. This signal may be inherent in the analyte or a result of a chemical reaction involving the analyte (such as in colorimetric methods). The UV-Vis portion of the electromagnetic spectrum spans from 200-700nm (Penner 2003).

1.3.2 Fourier-Transform Infrared Spectroscopy

In an excited state, molecules have specific frequencies at which their shape is altered, either through rotation or vibration. Infrared (IR) spectroscopy measures the absorption levels of these molecules at different infrared frequencies. The infrared region of the electromagnetic spectrum extends from 14000-50cm\(^{-1}\) and is divided into 3 areas: Far IR (400-50cm\(^{-1}\)), Mid IR (4000-400cm\(^{-1}\)) and Near IR (14000-4000cm\(^{-1}\)). Mid IR is
commonly used for structural identification (fingerprinting) of organic compounds because the absorption bands are due to vibration of a specific functional group (Guillen and Cabo 1997). The fingerprint region, located within the MIR region between 1200 and 700 cm\(^{-1}\), contains bands from lipids, proteins, carotenoids, and polysaccharides (Pare and Belanger 1997). Spectra from this region can be used for quantitative analysis applications because the intensities of the bands are proportional to the concentration of their respective functional group (Pare and Belanger 1997).

Infrared spectroscopic techniques have become increasingly popular tools for food analysis due to their rapid analysis time (Baeten and Dardenne 2002). The spectroscopic analysis of lipids is possible because these compounds show characteristic absorption bands in the Mid IR region (Guillen and Cabo 1997). While at first glance the spectra of different types of edible oils appear similar, there are distinct differences due to the intensity of bands at 3006, 1654, 1417, 1402, 1238, 1163, 1118, 1097, 914 and 723 cm\(^{-1}\). Extensive research has been done to match wavenumbers to functional groups (Guillen and Cabo 1998). For example, a band at 3006 cm\(^{-1}\) is due to =C-H stretching and one at 1163 cm\(^{-1}\) is due to either –C-O- or –CH\(_2\)- stretching and bending (Guillen and Cabo 1998).

Fourier Transform Infrared Spectroscopy (FTIR) technology has substantial potential as a quantitative QC tool in the food industry. Currently the technology is used for fat and moisture determination in butter, protein and fat content in raw meat, and determining alcohol content in beer (Guillen and Cabo 1997). The numerous advantages of FTIR over traditional dispersive infrared instruments make the equipment an excellent tool for quantitative analysis of edible oils. These advantages include: low wear on
equipment, improvement in the signal-to-noise ratio, simultaneous detection of all frequencies (reducing scan time without losing resolution), superior wavelength resolution, internal wavelength calibration, and advanced wavelength accuracy (Guillen and Cabo 1997). Since the wavelength of the laser is stable and very accurately known, the data can be acquired at precise pathlengths. Thus, the accuracy of the interferometric data is very high, allowing for repetitive scans to be well aligned with respect to each other. In FT instrumentation, the resolution is not determined by the size of the beam, but by the stroke (travel) of the movable mirror and the number of data collected during a stroke. These optical advantages result in a significantly reduced data acquisition time compared to a spectrum obtained with similar resolution on a dispersive instrument. These advantages more than compensate for the disadvantage that data has to be transformed mathematically to obtain the desired spectra (Diem 1993).

Unlike traditional dispersive IR spectrometers which use a monochromator to separate light into individual wavelengths, FTIR uses an interferometer to divide light. The interferometer contains a beam splitter which reflects one beam of light onto a stationary mirror and the remainder is reflected onto a moving mirror. The beam is recombined after reflecting off the respective mirrors, and is split again via the beam splitter. Following interference, the beam is passed through the sample and the signal is transmitted to the detector (Figure 4). The signal is converted by the Fourier Transform algorithm and a spectrum is produced. The spectrum is made up of a series of bands reflecting the absorbance of the different functional groups in a compound (Pare and Belanger 1997).
There are numerous reasons that IR spectroscopy is so widespread. The simplest reason is that the technique has been around longer than other techniques. In addition, sample preparation is easy and materials can be examined in the gaseous, liquid, or solid states. In the case of edible oils, no sample preparation is needed. Data acquisition takes only a few seconds and a qualitative interpretation is often possible via quick inspection of the spectral features. Finally, the price of these instruments is very reasonable, particularly compared to other available instruments such as nuclear magnetic resonance (Diem 1993).

One of the few disadvantages of IR spectroscopy is that glass does not transmit in the infrared region. As a result, all IR sample cells must be equipped with windows that are transparent in the spectral regions of interest. Crystals with different properties are
used for the windows. Zinc selenide (ZnSe), for instance, is a chemically inert, water insoluble crystal and is used in the region 5000-500cm\(^{-1}\). Potassium bromide (KBr) is hygroscopic and is also used in the Mid IR region (Diem 1993). It is important to use the correct crystal for the type of sample being analyzed in order to avoid damaging the sample cell.

Fourier transform infrared spectroscopy may be able to substitute classic oxidation indices in the determination of oxidative stability due to its simplicity, low cost and time savings. Changes observed in infrared data are useful indicators of edible oils oxidative stability and are closely related to changes observed in peroxide values in the course of oxidation of the samples (Guillen and Cabo 2002).

In one study, PTFE disposable IR cards were used as substrates to rapidly oxidize safflower, sunflower, and canola oils and to simultaneously monitor the extent of oxidation by FTIR spectroscopy (Russin et al. 2004). The small sampling area on the cards allowed for rapid oxidation of the samples at lower temperatures (58\(^\circ\)C). Most of the common methods for monitoring autoxidation and determining the oxidative stability of edible oils use elevated temperatures (above 98\(^\circ\)C) to accelerate the oxidation process, which often changes the mechanism of oxidation and makes it difficult to extrapolate the data to ambient storage conditions. From different oil samples, Russin (2004) looked at the peak height of the peroxide (ROOH) absorption band at 3444cm\(^{-1}\). The oxidation process was allowed to continue until peroxide values were above 300 mEq oxygen/kg oil. He found that the hydroperoxide absorption band starts at 3444cm\(^{-1}\) in spectra of oils with low peroxide values, but shifts to progressively lower wavenumbers as oxidation proceeds (Russin, van de Voort, Sedman 2004).
There have been attempts to automate the AOCS PV determination method with FTIR. Because FTIR systems require online computing capabilities, most units come equipped with powerful software that provides a way to process data and automate the procedure if an appropriate means of calibration can be devised. Because so many sources of interference and variability affect PV determination, researchers have used partial least squares regression (PLSR) with spectral analysis because it is one of the most powerful chemometric techniques available. PLSR is capable of accounting for interactions, underlying absorptions, overlapping bands, and other factors that may affect the spectra as the concentration of components change over time. While Van de Voort’s (1994) found this method had better overall reproducibility than chemical methods, development of a PV calibration is a complex undertaking and would be difficult for anyone not familiar with FTIR and PLSR. In order for the calibration to be successful in the food industry, the instrument must be preprogrammed so that the operator does not require any knowledge of FTIR and would be guided through analysis by prompts or instructions. Transferability of calibration is important so that the method can be implemented on different instruments without the need for individual calibration. So far, this has proven to be a very difficult task due to variation in instruments and operating systems (van de Voort and others 1994).

The clearest beneficiaries of FTIR methods that would replace traditional wet chemistry techniques would be laboratories that carry out edible oil quality analysis on the behalf of their respective industrial sectors. For these labs, speed, accuracy, and utility (the ability to use a single instrument to carry out a range of analyses) are important
criteria, as well as minimizing the solvent disposal problems that are part of most of the wet chemical methods still in common use (van de Voort and others 2008).

Figure 5: TruDefenderFT® infrared spectrometer

Source: Ahura Scientific, 2009

Several manufactures have recently developed handheld infrared spectrometers. Instruments such as the TruDefenderFT® come equipped with an ATR diamond crystal and a spectral range of 4000-650cm\(^{-1}\) (Figure 5). These handheld units are praised for their ability to bring the analytical precision of spectroscopy to field applications. Handheld infrared spectrometers have the potential to replace traditional wet chemistry techniques for the analysis of oils. They are fast (data collection takes less than two minutes), accurate, require a minimal amount of sample (40\(\mu\)L) and are easy to use. Most importantly, no solvents are required for oil sample preparation. The crystal can be cleaned with a small amount of ethanol in between samples.

1.4 CHEMOMETRICS

A combination of vibrational spectroscopy and multivariate statistical analysis has made it possible to extract important information from spectra. Principal component
analysis (PCA) is a multivariate projection method designed to show the variations within the data matrix and can be used to develop a modeling technique referred to as SIMCA (soft independent modeling of class analogy). SIMCA is one of the most commonly used class-modeling tools in chemometrics. With this method, PCA is performed for each class separately, resulting in a principal component (PC) model for each class (Gurdeniz and Ozen 2009). Each PC represents the main systemic variation in the data set which can be modeled after the extraction of the previous one (Dupuy and others 1996). A class space is built around the data representing a 95% confidence interval. The boundary of the space discriminates between samples that fit into the class model and samples that do not (Gurdeniz and Ozen 2009). New measurements are projected in each PC space to evaluate whether they belong (Maesschalck and others 1999). An advantage of models formed using SIMCA is that noise in the spectra is taken into consideration when developing the component spaces (Dupuy and others 1996). Using SIMCA’s discriminating power algorithm, it is possible to identify wavenumbers of interest and also to remove those that do not assist in separating classes (Dunn and Wold 1995). Interclass distances represent the Mahalanobis distance between classes based on factor loadings. Distances greater than or equal to three are considered significantly different. Interclass distances can be used to determine whether it is possible to completely separate the classes.

Partial Least Squares Regression (PLSR) is another commonly used chemometric technique. It models the relationship between two factors in a linear fashion. When applied to vibrational spectroscopy, one of the factors is the spectral data and the other can be any of a variety of reference methods such as PV. PLSR is a way to estimate
parameters in a linear scientific model (Wold, Sjostrom, Eriksson 2001). The strength of this chemometric technique is based on its ability to mathematically correlate spectral changes to changes in the concentration of a component of interest while simultaneously accounting for all other significant spectral factors that influence the spectrum (van de Voort and others 1994). The coefficient of correlation (r-value) and standard errors are used to evaluate the accuracy of the generated model.

Multivariate analysis combined with vibrational spectroscopy can be applied to analysis of oil oxidation and to determine important quality parameters.
1.5 REFERENCES


Farmer E, Bloomfield A, Sundralingam, Sutton D. 1941. The course and mechanism of autoxidiation reactions in olefinic and polyolefinic substances, including rubber.


Chapter 2

Use of FTIR for Rapid Authentication and Detection of Adulteration of Food

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2.1 ABSTRACT

Fourier Transform infrared (FTIR) spectroscopy is an appealing technology for the food industry because simple, rapid, and non-destructive measurements of chemical and physical components can be obtained. Advances in FTIR instrumentation combined with the development of powerful multivariate data analysis methods makes this technology ideal for large volume, rapid screening and characterization of minor food components down to ppb levels. Due to the use of FTIR techniques in quality and process control applications, the food industry is already familiar with the technology and the potential exists to expand its scope to monitoring for food adulteration. The aim of this review is to compile the current research on applications of near infrared (NIR) and mid infrared (MIR) spectroscopy for rapid authentication and detection of adulteration in food.

2.2 INTRODUCTION

Fourier Transform infrared (FTIR) spectroscopy has become an attractive alternative for traditional tedious analytical methods because little sample preparation is needed, analysis is rapid, and the use of hazardous solvents is minimized. These advantages result in dramatic time and cost savings and increase the number of samples that can be analyzed. Numerous researchers have attempted to utilize these advantages by applying FTIR to food science. The scope of this review is to compile the current research on the applications of FTIR spectroscopy for authentication and detection of adulteration in food.

Knowledge of the basic principles behind spectroscopy facilitates understanding how the infrared technique can be applied to authenticate and detect adulteration in food.
Infrared spectra are produced by recording changes in absorption of IR radiation by molecules, which experiences mechanical motions (vibrational and rotational modes) due to the absorption of energy (Diem 1993; Guillen and Cabo 1997). Absorption of infrared radiation at different wavenumbers corresponds to specific functional groups, many of which have been characterized (Diem 1993). Thus, the IR spectra can be used to identify or differentiate between samples and also give information about the quantity of functional groups (Diem 1993; Guillen and Cabo 1997). The infrared region of the electromagnetic spectrum spans from 14000-50 cm\(^{-1}\) and is divided into 3 areas: Near IR (14000-4000 cm\(^{-1}\)), MidIR (4000-400 cm\(^{-1}\)), and Far IR (400-50 cm\(^{-1}\)). This review will focus on the first two regions. Near IR (NIR) and MidIR (MIR) techniques take advantage of the phenomenon occurring when electromagnetic radiation of a specific energy interacts with a molecule.

NIR spectra (Figure 6) are the result of relatively weak and broad overtone and combination bands of fundamental vibrational transitions associated mainly with C-H, N-H, and O-H functional groups. NIR bands are the result of complex vibrational movements of chemical bonds that tend to deviate from harmonicity. These deviations (anharmonicity) result in bands arising from transitions to higher energy levels with double, triple, etc. the frequency of fundamental vibrations (overtones) and bands from the interaction of two or more vibrations taking place simultaneously (combination bands) (Osborne 2000; Barton II 2002). NIR bands are 10-100 times less intense than their corresponding mid-infrared fundamental bands. This can allow for the direct analysis of samples that are highly absorbing or strongly light scattering without dilution or extensive sample preparation (Shenk, Workman, Weterhaus 2001; Hall and others
FT-NIR instruments have found a niche in the food industry because of their ruggedness and increased flexible handling options such as being able to analyze samples in glass vials. Furthermore, the relatively weak absorption due to water overtones enables high-moisture foods to be readily analyzed (Osborne 2000).

Figure 6: Near- (A.) and mid-infrared (B.) raw spectra of grapeseed oil

Spectra from the Mid IR region are commonly used for structural identification (fingerprinting) of organic compounds because the absorption bands are due to fundamental vibrations of a specific functional group (Guillen and Cabo 1997). The fingerprint region, located within the MIR region between 1200 and 700 cm\(^{-1}\), contains bands from lipids, proteins, carotenoids, and polysaccharides and as a result is rich in structural information (Pare and Belanger 1997). Spectra from the MIR region (Figure 6B) can be used for quantitative analysis applications because the intensities of the bands
are proportional to the concentration of their respective functional group (Pare and Belanger 1997).

With the advent of Fourier-Transform instrumentation, IR technology has greatly increased its speed of analysis and accuracy by replacing the use of conventional prism and monochromators. Fourier-Transform infrared spectroscopy (FTIR) utilizes interferometric modulation of radiation to measure multiple frequencies simultaneously producing an interferogram that is recalculated using complex algorithms to give the original spectrum. Fourier deconvolution resolves overlapping IR bands, caused by complex samples, by reducing the bandwidth and increasing the peak height (Markovich 1991). Additional advantages of FTIR over traditional dispersive infrared instruments include: low wear on equipment, improvement in the signal-to-noise ratio, superior wavelength resolution, internal wavelength calibration, and advanced wavelength accuracy (Guillen and Cabo 1997). Since the wavelength of the laser is stable and very accurately known, the data can be precisely acquired, allowing for repetitive scans to be well aligned with respect to each other. In FT instrumentation, the resolution is not determined by the size of the beam, but by the stroke (travel) of the movable mirror and the number of data collected during a stroke. These optical advantages result in a significantly reduced data acquisition time compared to a spectrum obtained with similar resolution on a dispersive instrument (Diem 1993) making the technology an excellent tool for qualitative and quantitative analysis of food matrices.

The development of attenuated total reflectance (ATR) has made an important contribution to the FTIR technique by allowing the spectral collection of solids, liquids, semisolids and thin films. ATR provides a fast analytical tool because less sample
preparation required, improves the sample to sample reproducibility, and gives high throughput relative to the available energy in the FTIR sample compartment, resulting in better quality data (Pike Technologies 2010). ATR is a reflection technique where the IR light is reflected internally off the back surface of internal reflection element with high index refraction, which is in contact with the sample (PerkinElmer 2004). The infrared beam travels inside the crystal and then a standing wave of radiation is created, called the evanescent wave (PerkinElmer 2004). A sample in contact with the crystal can interact with the evanescent wave, absorb infrared radiation and have its infrared spectrum detected. The evanescent wave is attenuated by the sample’s absorbance giving rise to the name attenuated total reflectance (PerkinElmer 2004). The high refractive index crystals typically are made of diamond, zinc selenide, KRS-5 (thallium iodide/thallium bromide), or germanium. The number of reflections at the surface of the crystal will vary depending on length and thickness of the crystal and the angle of incidence (PerkinElmer 2004). This provides the ATR with multiple fold increase in sample’s response compared to single-reflection crystals (Pike Technologies 2010).

Several excellent books cover aspects of fundamental theory, instrumentation, chemometric methods and applications of vibrational spectroscopy and should be referenced if more detailed information is desired (Chalmers and Griffiths 2001; Robinson 1991; Sielsler and others 2002; Williams and Norris 2001; Osborne 2000).

2.3 APPLICATIONS OF FTIR FOR AUTHENTICATION AND DETECTION OF ADULTERANTS

Authentication of products by commodity, variety and geographical origin is important for regulatory agencies, food processors, retailers and consumers since
expensive ingredients can be subject to adulteration and fraudulent or accidental mislabeling. There is a need for a rapid technique to validate these claims and the potential application of FTIR has been explored in recent years. Combining FT-NIR and FT-MIR with multivariate statistical methods has been applied for authentication of herbal products, fruit juices, agricultural products, edible oils, dairy, and numerous other food products. These efforts have had varying degrees of success at classifying products as authentic or unauthentic depending on the region of the electromagnetic spectrum employed and chemometric techniques used on the spectra. Fingerprints of authentic commodities may be considered to represent their overall chemical composition and therefore have the potential to detect adulteration. This method of detection possesses various benefits as an authenticity screening tool: it is extremely fast (tests can be carried out in 1-2 min), simple to use, and may be implemented by unskilled personnel. Further, a large number of potential adulterants may be searched from single spectra, no sample preparation is required, and little waste material is generated. From a regulatory perspective, it has the additional benefit of not destroying the sample under test (Kelly and Downey 2005).

Table 2 summarizes the applications of NIR spectroscopy in monitoring authentication of foods. NIR reflectance spectroscopy has been used to develop a fast authentication system for herbal supplements. *Echinacea* species, *E. purpurea* (L.) Moench, *E. angustifolia* and *E. pallida*, are widely used as immuno-stimulant herbal preparations and commercial preparations are frequently adulterated or substituted with roots of *Parthenium integrifolium* L., or different *Echinacea* species which negatively affect the reliability and efficacy of *Echinacea* commercial products (Laasonen et al.
NIR spectroscopy has been reported for the fast identification of *Echinacea purpurea* roots (Laasonen and others 2002b) and the determination of echinacoside content (Schulz and others 2002). The presence of other *Echinacea* species could be detected at a minimum of 10% adulteration by using FT-NIR spectroscopy (Laasonen and others 2002b). Due to the low content of echinacosides in the most valuable *E. purpurea* roots as compared to *E. pallida* and *E. angustifolia* (Laasonen and others 2002b), PLSR algorithms using NIR spectra produced robust models for the fast and reliable screening of *E. purpurea* in herbal preparations.

Adulteration of dietary supplement oils (DSO: grapeseed, flax, evening primrose, and others) with cheaper and less beneficial oils has become a food quality/safety issue. Variations between different brands of the same oil due to plant origin, variety, and processing conditions, as well as oil types having very similar compositions, can result in possible misclassifications of authentic oils. A detection limit of 2% for DSO adulterated (2-20% v/v) with common foods oils has been reported (Ozen, Weiss, Mauer 2003).

A noteworthy application of NIR spectroscopy has been for the detection of adulterants in juices, purees and syrups. These products are adulterated with cheaper juice concentrates, cane, corn or beet sugars and syrups for economic gain. Twomey et al. (1995) reported the use of NIR and factorial discriminant analysis for the detection of adulteration of orange juice with orange pulpwash, grapefruit juice, and synthetic sugar/acid mixture.
Table 2: Application of Dispersive NIR for food authenticity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Multivariate model</th>
<th>Results</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinacosides in Echinacea roots</td>
<td>Reflectance</td>
<td>PLSR</td>
<td>$R^2 = 0.94$, RMSECV = 0.23 g/100g</td>
<td>Schulz et al., 2002</td>
</tr>
<tr>
<td>Adulteration of Orange Juice</td>
<td>Reflectance</td>
<td>FDA</td>
<td>94% accuracy at levels &gt;50 g/kg adulterants</td>
<td>Twomey et al., 2004</td>
</tr>
<tr>
<td>Cocoa Procyanidins</td>
<td>Reflectance</td>
<td>PLSR</td>
<td>$R^2 = 0.98$, SECV = 6.20</td>
<td>Whitacre et al., 2003</td>
</tr>
<tr>
<td>Phenolic substances and alkaloids in green tea leaves</td>
<td>Reflectance</td>
<td>PLSR</td>
<td>Gallic acid, SECV: 0.2 g/kg, $R^2$: 0.89; Epicatechin, SECV: 2.6 g/kg $R^2$: 0.97; Caffeine, SECV: 1.7 g/kg, $R^2$: 0.97</td>
<td>Schulz et al., 1999</td>
</tr>
<tr>
<td>Persettol in Avocado Honey</td>
<td>Reflectance</td>
<td>PLSR, PCR</td>
<td>$R^2 = 0.87$, SEP = 0.13</td>
<td>Dvash et al., 2002</td>
</tr>
<tr>
<td>Citrus Oils</td>
<td>Reflecance</td>
<td>PCA, PLSR</td>
<td>$R^2 = 0.79 - 1.00$, SEC = 0.03 - 1.09</td>
<td>Steuer et al., 2000</td>
</tr>
<tr>
<td>Apple Adulteration in Strawberry and raspberry purees</td>
<td>Reflectance</td>
<td>SIMCA, PLSR</td>
<td>Most accurate models produced prediction errors of 3.4% apple (in raspberry) &amp; 5.5% (in strawberry)</td>
<td>Contal et al., 2002</td>
</tr>
<tr>
<td>Vegetable Proteins in Milk Powder</td>
<td>Reflectance</td>
<td>MLR</td>
<td>$R^2 = 0.99$, SEP = 0.23</td>
<td>Maraboli et al., 2002</td>
</tr>
<tr>
<td>Authentication of Green Asparagus</td>
<td>Reflectance</td>
<td>PLSR</td>
<td>$R^2 &gt; 0.96$, SEP = 0.07</td>
<td>Perez et al., 2001</td>
</tr>
<tr>
<td>Adulteration in alcoholic beverages</td>
<td>Transmittance</td>
<td>PCA, SIMCA</td>
<td>correct classification of 100%</td>
<td>Pontes et al., 2006</td>
</tr>
<tr>
<td>(Online) acrylamide adulteration in chips</td>
<td>Reflectance</td>
<td>PLSR</td>
<td>$R^2 = 0.83$; prediction error 266.6ug/kg (using low resolution equipment)</td>
<td>Pedreschi et al., 2010</td>
</tr>
<tr>
<td>Acrylamide adulteration in chips</td>
<td>Reflectance</td>
<td>PLSR</td>
<td>$R^2 = 0.95$, prediction error 256.6ug/kg</td>
<td>Segtnan et al., 2006</td>
</tr>
<tr>
<td>Whey adulteration in cow milk</td>
<td>Reflectance</td>
<td>DPLS, SIMCA</td>
<td>$R^2 = 0.999$, RMSEP = 0.264</td>
<td>Kasemsumran et al., 2007</td>
</tr>
</tbody>
</table>

PLSR: Partial Least Squares Regression
PCA: Principal Component Regression
SIMCA: Soft Independent Model Class Regression
LDA: Linear Discriminant Analysis Regression
MLR: Multiple Linear Regression
Table 3: Application of MIR for food authenticity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Multivariate model</th>
<th>Results</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lard adulteration in cake</td>
<td>PLSR</td>
<td>$R^2=0.9790$  SEC=1.75</td>
<td>Syahariza et al., 2005</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Lard adulteration in chocolate</td>
<td>PLSR</td>
<td>$R^2=0.99$  SE=1.30</td>
<td>Che Man et al., 2003</td>
</tr>
<tr>
<td>Extra virgin olive oil Adulteration with</td>
<td>PLSR</td>
<td>$R^2=0.99$; detection limit 6%</td>
<td>Vlachos et al., 2006</td>
</tr>
<tr>
<td>vegetable oils</td>
<td>PCA</td>
<td>detection limit of 5% for binary mixture,</td>
<td>Gurdeniz and Ozen, 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>error limit 1.04</td>
<td></td>
</tr>
<tr>
<td>Adulterated with palm oil</td>
<td>PLS and PCR</td>
<td>$R^2=0.999$, SECV=0.285 (1st deriv.)</td>
<td>Rohman and Che Man, 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>was able to correctly classify 80%</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(mean centered and 1st and 2nd deriv.)</td>
<td></td>
</tr>
<tr>
<td>Evaluating origin</td>
<td>PLS</td>
<td></td>
<td>Hennessy et al., 2009</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juice concentrate adulteration</td>
<td>PLSR</td>
<td>$R^2=0.9751$; could also predict total</td>
<td>Vardin et al., 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>solids ($R^2=0.9916$) and titratable</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>acidity ($R^2=0.9114$)</td>
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<tr>
<td></td>
<td></td>
<td>extraction improved SIMCA, 100% correct</td>
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<tr>
<td></td>
<td></td>
<td>classification at commodity level</td>
<td></td>
</tr>
<tr>
<td>Authentication of fruits</td>
<td>SIMCA</td>
<td>100% classification of simple and complex</td>
<td>He et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sugars using PLS and LDA; combining honey</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>varieties lowered it to 95.5%</td>
<td>Sivakesava and Irudayaraj, 2002</td>
</tr>
<tr>
<td>Classifying honey adulterants as simple</td>
<td>LDA, PCA, LDS</td>
<td>classification over 95% for beet sucrose</td>
<td></td>
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<tr>
<td>and complex sugars</td>
<td></td>
<td>and dextrose, but couldn’t unambiguously</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>detect HFCS or invert beet</td>
<td>Sivakesava and Irudayaraj, 2002</td>
</tr>
<tr>
<td>Artisinal honeys adulterated with sugar</td>
<td>SIMCA, PLSR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>solutions</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Butter adulterated with margarine</td>
<td>PLSR</td>
<td>$R^2=0.99$, SECV= 1.2% (2nd deriv.)</td>
<td>Koca et al., 2010</td>
</tr>
<tr>
<td>Classifying wines as organic versus</td>
<td>PCA, DPLS, LDA</td>
<td>using calibration models of selected ranges</td>
<td></td>
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<tr>
<td>nonorganic</td>
<td></td>
<td>(0-5%, 0-25, 20-60, etc)</td>
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<tr>
<td></td>
<td>DPLS correctly</td>
<td></td>
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<tr>
<td></td>
<td>classified 85%</td>
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<tr>
<td></td>
<td>LDA correctly</td>
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<tr>
<td></td>
<td>classified 75%</td>
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<td></td>
</tr>
</tbody>
</table>

PLSR: Partial Least Squares Regression
SIMCA: Soft Independent Model Class Analogy
PCA: Principal Component Analysis
PCR: Principal Component Regression
CVA: Canonical Variate Analysis
LDA: Linear Discriminant Analysis
Accurate classification rates >90% were determined for adulterated orange juice at 50g/kg or higher levels, with no adulterated orange juice being predicted as authentic. Contal et al. (2002) showed that adulteration of strawberry or raspberry juice with apple juice could be detected at levels >10% by using PLS-NIR models. Although NIR measurements of aqueous systems have been difficult because of the interference from broad vibrational bands of water (Fischer and others 1994), the strong effect of water signals can be minimized by rapid solvent elimination and measuring the dry extract by diffuse reflectance spectroscopy (Li, Goovaerts, Meurens 1996; Alfaro, Meurens, Birth 1990) and by using the derivatized spectra on transmittance or transreflectance measurements (Rodriguez-Saona and others 2001; Segtman and Isaksson 2000). Transmittance spectra can accurately and precisely predict the sugar levels in non-scattering juices (Rodriguez-Saona and others 2001) while NIR transreflectance data improve the prediction errors for scattering juice samples (Segtman and Isaksson 2000).

Dvash et al. (2002) reports an interesting application of NIR for the determination of perseitol, a sugar that is specific to avocado honey. NIR analysis was used to detect to what extent honeybees have harvested avocado nectar. Although the average concentration of perseitol in honey samples was only 0.48%, the calibration models gave standard error of prediction (SEP) and $R^2$ values of 0.13% and 0.87, respectively. Only honeys in the highest 20% range of perseitol content could be unambiguously classified as avocado honey. FT-NIR spectroscopy could be easily used as a quality control measure to assess for adulteration of beverages.

Table 3 summarizes the applications of MIR spectroscopy in monitoring authentication of foods. MIR has been used in juice authentication of high-value
ingredients adulterated with inferior sources. For instance, pomegranates have been praised for their antioxidant activity and for potential chemopreventative effects against prostate cancer (Vardin and others 2008). MIR spectra have allowed the differentiation of pure pomegranate juice concentrate from juice adulterated with grape juice concentrate (2 to 14% v/v) using PCA and the 1780-1685 cm\(^{-1}\) (C=O stretching) infrared region (Vardin and others 2008). Similarly, MIR has been successful in differentiation of fruit varieties and geographical origins. An important parameter for monitoring authenticity in fruit purees, preps, and jams is the percent fruit content and minimum requirements for each product type have been established. PLSR correlating fruit content and FTIR spectra centered on a band at 1729 cm\(^{-1}\) provided good calibration statistics (R\(^2\)=0.94) when applied to strawberry jam (Fugel, Carle, Schieber 2005). He and others (2007) looked at cranberries, blueberries, concord grapes, plum nectar blend, and apple juices from various manufacturers. Spectral data collected after solid phase extraction of juices improved the pattern recognition (SIMCA) modeling power compared to using pure juice and allowed for differentiation of juices with varying origins. Solid phase extraction minimized the interference of sugar on the spectra and isolated the phenolic components that provided a unique fingerprint for juice authentication. The authors acknowledge the limitations of this method, including the lengthy extraction procedure and the need for a broader range of samples to improve the robustness of the model, but feel this method was a considerable improvement over previous attempts (He, Rodriguez-Saona, Guisti 2007). Traditional methods for authenticating fruit juices include chromatography or carbon isotope ratio analysis--neither of which is very practical because they are time-
consuming, not efficient enough for quality control settings, use harmful solvents, and they monitor only one parameter at a time.

In recent years, the food industry has noticed a shift in consumer perception of processed foods. While convenience is still an important quality, consumers are becoming more interested in organic products and are willing to pay a premium price for these items. The wine industry has acknowledged this trend and vineyards around the world are currently producing many organic wines. As of 2009, there was no standardized wine industry method that would enable organic wine composition and authenticity to be easily and efficiently determined (Cozzolino and others 2009). Cozzolino (2009) was the first to look at using MIR technology to classify commercial wines from organic versus non-organic production systems. Nearly 200 samples of red and white wines from 13 regions in Australia were analyzed using MIR combined with principal component analysis (PCA), discriminant PLS (DPLS) regression and linear discriminant analysis (LDA). DPLS correctly classified 85% of organic wines while LDA was able to classify 75%. In general, the PCA score plot separated the organic or non-organic wine classes, but there was a slight overlap (Cozzolino and others 2009).

Maple syrup and honey products are also targets for unscrupulous manufacturers to make profit by adding cheaper cane and beet sugars. NIR and FTIR spectroscopy in combination with discriminant (LDA and canonical variate analysis) and quantitative (PCR and PLSR) analysis has been successfully applied for the classification of adulterants in maple syrup (Paradkar, Sivakesava, Irudayara 2003). Models developed with NIR measurements were suited for quantitative analysis of the presence of adulterants (Table 4).
**Figure 7:** Classification of adulterants in maple syrup by NIR (A) and MIR (B) using PLS/CVA

**Table 4:** Model validation of FT-NIR and mid-infrared methods for the determination of food components

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Factors</th>
<th>SECV</th>
<th>R²</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C in Foods and Pharmaceuticals</td>
<td>FT-IR-Attenuated Total Reflection</td>
<td>11</td>
<td>0.28%</td>
<td>0.998</td>
<td>Yang and Irudayaraj, 2002</td>
</tr>
<tr>
<td></td>
<td>NIR Diffuse Reflectance DRIFTS</td>
<td>9</td>
<td>1.93%</td>
<td>0.975</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FT-IR Purged Photoacoustic detector</td>
<td>9</td>
<td>1.45%</td>
<td>0.985</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NIR reflectance fiber optic probe</td>
<td>5</td>
<td>1.23%</td>
<td>0.973</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FT-NIR reflectance</td>
<td>3</td>
<td>1.64%</td>
<td>0.980</td>
<td></td>
</tr>
<tr>
<td>Cholesterol in dairy products</td>
<td>FT-NIR</td>
<td>2</td>
<td>1.44%</td>
<td>0.99</td>
<td>Parakar and Irudayaraj, 2002a</td>
</tr>
<tr>
<td></td>
<td>FT-mid infrared - 1st derivative</td>
<td>1</td>
<td>0.68%</td>
<td>1.00</td>
<td>Parakar and Irudayaraj, 2002b</td>
</tr>
<tr>
<td>Tetracycline in Milk (range 4-2000 ppb)</td>
<td>FT-NIR</td>
<td>4</td>
<td>450 ppb</td>
<td>0.72</td>
<td>Sivakesava and Irudayaraj, 2002</td>
</tr>
<tr>
<td></td>
<td>FT- mid infrared</td>
<td>15</td>
<td>382 ppb</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Tetracycline in Milk (range 4-520 ppb)</td>
<td>FT-NIR</td>
<td>4</td>
<td>110 ppb</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FT- mid infrared</td>
<td>11</td>
<td>101ppb</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Extra virgin olive oil adulteration</td>
<td>FT-NIR</td>
<td>13</td>
<td>3.48%</td>
<td>0.99</td>
<td>Yang and Irudayaraj, 2001</td>
</tr>
<tr>
<td></td>
<td>FT- mid infrared</td>
<td>11</td>
<td>4.74%</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Infant formula adulterated with melamine</td>
<td>FT-NIR</td>
<td>6</td>
<td>(RMSECV)0.62</td>
<td>0.99</td>
<td>Mauer et al., 2009</td>
</tr>
<tr>
<td></td>
<td>FT-mid infrared</td>
<td>10</td>
<td>(RMSECV) 1</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Maple syrup</td>
<td>FT-NIR</td>
<td>11</td>
<td>3.872</td>
<td>0.95</td>
<td>Parakar et al., 2003</td>
</tr>
<tr>
<td></td>
<td>FT-mid infrared (region 800-1200 cm⁻¹)</td>
<td>6</td>
<td>2.091</td>
<td>0.97</td>
<td></td>
</tr>
</tbody>
</table>

SECV=Standard error cross validation
Models developed from FTIR spectra using the fingerprint region resulted in models with superior quantitative and discriminative performances (Table 4, Figure 7) for detecting adulterants as compared to those obtained from dispersive NIR spectra. Similar results have been reported for the analysis of thyme, oregano and chamomile essential oils by dispersive NIR and ATR-IR spectroscopy (Schulz and others 2003).

Honey has been defined as a natural substance produced by honey bees and the addition of sugars violates this definition (Kelly and others 2006). Stretching honey with simple and complex sugars is the most consistent way honey is adulterated. This has been very difficult to detect because the adulterants mimic the natural sugar profile of honey (38.2% fructose and 31.2% glucose)(Sivakesava and Irudayaraj 2002a). Honey adulteration is also difficult to detect because of the large variability in the product due to flower and bee species, maturity, environment, and processing or storage conditions. Purity is currently tested by carbon isotope ratio analysis which is expensive and time consuming. In order to use MIR to authenticate honey, the spectra needed to be corrected against a background of water to correct for water overlapping the signal from solutes. Analysis was focused on the region that corresponds to sugars (800-1500 cm\(^{-1}\)). Pure honey was adulterated with 7-25% glucose, fructose, sucrose, and invert sugar. LDA achieved 100% classification of simple sugars and PLS data compression achieved 100% classification for complex sugars. Combining varieties of honey required more factors and lowered the success rate to 95.5%. Further work must be conducted to include honey of many origins and with many adulterants (Sivakesava and Irudayaraj 2002a).

A similar study looked at artisanal honey adulterated with different sugar syrups (invert beet syrup, HFCS, partial invert cane syrup, dextrose syrup and beet sucrose).
Models were able to correctly classify 95% of authentic honey, beet sucrose, and dextrose samples, but were not able to confidently detect adulteration with HFCS or invert beet syrup (Kelly, Petisco, Downey 2006). Iglasias (2006) used MIR to evaluate the botanical origin of honey when correlated with pollen analysis. As with many food products, honey produced in certain regions is prized for its outstanding sensory qualities and comes with a high price. MIR could be used as a screening tool but would need to be combined with additional testing to confidently identify origin of the product. Furthermore, MIR did not allow for a quantitative determination of hydroxymethyl furan (HMF) as an indicator of heat damage to the product (Iglasias 2006).

FTIR spectroscopic has shown potential to determine lard adulteration. Syahariza (2005) evaluated lard adulteration in shortening at levels of 0-100% combined with PLSR models in the regions 1117-1097 and 990-950 cm\(^{-1}\), producing a model with an \(R^2=0.98\) and a standard error of calibration of 1.75. Also, ATR-IR combined with PLSR regression was able to detect cocoa butter spiked with lard (0-15%) with an \(R^2=0.99\) and a standard error of 1.305 (CheMan and others 2005).

Adulteration of olive oil dates back at least to the time of the Roman Empire (Ulberth and Buchgraber 2000). Applying spectroscopy for olive oil authentication emerged in the mid 1990’s and a considerable amount of work has been devoted to using the technique for detection of extra virgin olive oil (EVOO) adulteration, specifically for improving limits of detection. The band at 3009 cm\(^{-1}\) has been identified for quantification of adulteration because the height of this band for EVOO is smaller than it is for other types of oils and changes according to the extent of adulteration. High correlation coefficient (above 0.99) was established with a detection limit of
approximately 6% (Vlachos and others 2006). Wavelet compression prior to PCA produces a detection limit of 5% for binary oil mixtures (Gurdeniz and Ozen 2009). EVOO adulteration with palm oil can be detected using first derivative of the FTIR spectra in the fingerprint region and PLSR ($R^2=0.999$, SECV= 0.285) (Rohman and Che Man 2010). FTIR spectra reflect different substitution patterns of triglycerides, differences in chain length of acyl moieties, and differing degrees of unsaturation (Guillen and Cabo 1997).

EVOO has also been the subject of studies to confirm geographical origin claims. The European Union supplies EVOO manufacturers with labels assigning protected designation of origin. NMR has been used to classify samples with up to 90% success, but this method is expensive and time consuming. Mean centered first and second derivative spectra combined with PLSR correctly classified 80% of samples on the basis of origin. This method is ideal for screening because of the high throughput (Hennessy, Downey, O’Donnell 2009).

The high demand for dairy products in the United States has resulted in premium prices for these products. Traditional methods for monitoring milk authenticity rely on wet chemistry to determine the amount of a marker compound in a suspect material and a subsequent comparison of values obtained with those from an equivalent material (Karoui and Baerdemaeker 2007). Woodcock and others (2008) reviewed the current state of development of both near-infrared (NIR) and mid-infrared (MIR) in cheese authenticity with ATR-IR spectroscopy being widely employed. These techniques are an emerging research area and have the potential to assist food processors to adhere to increasingly stringent food authenticity legislation. Picque and others (2002) reported the
discrimination of Emmental cheeses from different regions by IR spectroscopy. Data from ATR-IR spectroscopy of a water-soluble fraction enabled the classification of the cheeses with 78% accuracy, while 87% accuracy was obtained by using transmission spectra of dried extracts. The authors highlighted the ability of IR to discriminate according to the nature of the milk used for the manufacturing process. In another study, Pillonel and others (2002) reported the results of a broad screening test into the authenticity of Emmental cheese and its geographic traceability. NIR diffuse reflectance gave 100% discrimination by grouping into the six regions of cheese origin, whereas mid-IR transmittance achieved 100% correct classification when comparing “Switzerland” with the other regions pooled as one group.

The standard of identity of butter requires that the product contain no vegetable oil. Therefore the addition of margarine violates this claim. Current techniques for authenticating butter include GC, MS, NMR, and UV/Vis. These methods have detection limits of 2-5% but they are expensive and tedious. Infrared spectroscopy combined PLSR generated models that estimated adulteration of butter with levels of margarine ranging from 0-100% v/v with an $R^2>0.99$ and SECV<1.2%. Development of chemometric models using smaller ranges of adulteration levels (0-5%, 0-25%, and 20-60%) improved the robustness of the models (Koca and others 2010).

2.4 APPLICATIONS OF FTIR FOR DETECTION OF POTENTIALLY HARMFUL ADULTERANTS

Food products are most commonly adulterated with materials that are of a lower quality and, as a result, are typically less expensive. A smaller segment of adulteration includes products contaminated with potentially harmful compounds. This includes
compounds that unintentionally occur as a result of a production process (such as trans-fat and acrylamide) and intentional chemical contaminants (i.e. melamine). Advances in FTIR spectroscopic instrumentation and multivariate data analysis techniques show significant potential for determining changes in food composition that may be indicative of the addition of harmful extraneous material.

The trans-fat content of food has recently been identified as a health concern for the public. While small amounts of trans-fats are found naturally in dairy and meat products, the major source is partially hydrogenated vegetable oils used in commercial food products (Mossoba, Moss, Kramer 2009). The hydrogenation process is beneficial in that it enables oils that are low in saturated fat to be used in place of saturated fats, but partial hydrogenation produces trans-fats. Trans fatty acids have been documented to increase low density lipoprotein (LDL) cholesterol and lower high density lipoprotein (HDL), increasing the risk for coronary heart disease (McCarthy, Barr, Sinclair 2008). Beginning in 2006, the amount of trans-fat present in food and dietary supplements is required on the nutrition label and is to be expressed as g/serving (Mossoba, Moss, Kramer 2009). Amounts below 0.5g are recorded as trans-free. It is the FDA’s policy that it is the manufacturer’s responsibility to ensure the validity of a product’s stated nutrition information. Further, they define a product as misbranded if the amount of trans-fat found during FDA analysis is greater than 120% of what is presented on the nutrition label (Mossoba, Moss, Kramer 2009). The basic procedure for analysis via GC involves extracting the fat, preparing volatile fatty acid methyl ester (FAME) derivatives, resolving the mixture with a column that is capable of separating all the FAME components, summing all individual trans-FAME, correcting the detector’s response, and
finally converting to triacylglycerol equivalents (Mossoba, Moss, Kramer 2009). Because each step needs to be quantitative or representative, these methods require highly trained technicians.

IR methods have been used since the 1950’s to determine the amount of isolated trans double bonds in fats and oils. This method is based on the IR band at 966 cm\(^{-1}\) corresponding to CH- out of plane deformation (Figure 3A). This band is unique to isolated trans double bonds. The challenge with using this technique includes resolving the trans band from those due to conjugated double bonds or due to interferences attributed to other functional groups. It has been found that fats and oils with low levels of trans fatty acids are affected the most by overlapping bands. The trans band is found on an elevated sloping baseline, decreasing the accuracy of area or height measurements as the level of trans-fat decreases (Mossoba, Moss, Kramer 2009). Two ATR-FTIR official methods (AOCS Cd 14d-99 and AOAC method 2000.10) incorporate a background of trans-fat free oil to flatten the sloping baseline. Reference standards are created using trielaidin added to a trans-free reference oil. The standards are scanned on a 65°C single or multi-bounce ATR cell to generate a calibration curve. Using the curve, unknown trans levels, expressed as percent of total fat, can be calculated. It should be noted that using reference oil that differs considerably from the composition of the unknown sample can have an adverse affect on the accuracy of the model, especially below 5% total fat (Mossoba, Moss, Kramer 2009). A new negative second derivative ATR-FTIR method claims to improve accuracy and precision of quantitating levels of trans-fat in a food sample and is currently being validated by an international study. This method measures the height of the negative second derivative of trans absorption relative...
to air (Figure 8). Reference standards are generated from trans monoene trielaidin diluted in tripalmitin. This rapid method is ideal for determining total trans content that is needed for current labeling requirements (Mossoba, Moss, Kramer 2009).

Near infrared spectroscopy can also be used to determine of trans content in edible fats and oils. Li and others (1999, 2000a, 2000b) and Cox and others (2000) have done extensive work on the use of FT-NIR for the rapid determination of important quality parameters of fats and oils such as peroxide value, iodine value cis and trans content, and saponification number. Li and others (2000c) developed a PLS calibration model from FT-NIR spectra for the rapid determination of trans fats and oils. The calibration model was correlated to trans values determined by using MIR with single bounce horizontal attenuated total reflectance (IR-HATR) reference method (American Oil Chemist’s Society official method). There was no discernible trans absorption band in the FT-NIR spectrum as compared to the strong trans signal at 966 cm$^{-1}$ in the mid-IR spectrum. Nevertheless, the PLS-FT-NIR model was able to estimate the trans content of
edible oils. By using a training set that included a wide variety of oil types, the calibration model predicted the trans content with an accuracy of ± 1.1%. It was possible to obtain more accurate and reproducible predictions (± 0.5%) by calibrating a more limited training set that had specific characteristics. It is important to note that the reproducibility of the IR-HATR method is ± 0.4%. The product specific calibration produced serious predictive errors when non-representative samples were analyzed (Li and others 2002b).

Acrylamide is a maillard reaction product formed during baking, frying, and roasting foods such as potatoes and has recently been identified as a potential carcinogen. Standard procedures for acrylamide determination are based on chromatography and mass spectroscopy and are challenging to implement at manufacturing facilities for routine analysis. One acrylamide precursor, asparagine, is present in high levels in potatoes. Coupled with the high temperature/short time frying conditions results in potato chips having one of the highest known levels of acrylamide (Segtman and others 2006). NIR spectral analysis has focused on the bands originating from carbohydrates with a starch band at 1934nm found to be most significant. PLSR models using the spectra of ground chips in the region 400-2498nm correlated against predetermined quantities of acrylamide (R^2=0.95, prediction error of 256.6ug/kg). NIR spectral models were accurate enough for screening of acrylamide contents in processed potato crisps (Segtman and others 2006). However, the method needs to be tested and calibrated for each specific production process. Evaluation of the feasibility of using online monitoring of acrylamide in chips using NIR gave a model with a R^2=0.83 and prediction error of 266 ug/kg. The lower correlation was attributed to lower spectral resolution of the on-line instrument. On-line NIR monitoring could be used to separate samples with very high levels of
acrylamide from samples with average to low content (Pedreschi, Segman, Knutsen 2010).

The feasibility of using FT-NIR and multivariate analysis for the detection of food tampering with threat agents (Rodriguez-Saona, Fry, Calvey 2000) was developed and evaluated for the rapid detection of castor bean meal (CBM). The seeds of the castor plant (*Ricinus comunis*) contain the extremely toxic protein ricin that specifically and irreversibly inactivates eukaryotic ribosomes, promoting cell death by inhibiting protein synthesis. Castor bean meal is a by-product of the production of castor oil and is readily available and could easily be used to deliberately contaminate the food supply, thus making it a potential threat (Wellner, Hewetson, Ploi 1995). Analysis of spiked food matrices (bleached flour, wheat flour and blueberry pancake mix) with different CBM (0.5-8% w/w) levels by diffuse reflectance FT-NIR predicted the CBM contamination with standard error of cross-validation (SECV) < 0.6% and coefficient of correlation greater than 94%. Prediction of the CBM content by the calibration models was largely influenced by the spectral bands characteristic of amides (4880 and 4555 cm\(^{-1}\)) and lipids (5800, 5685, 4340 and 4261 cm\(^{-1}\)). PLSR models accurately predicted the content of CBM in contaminated samples with no false positives for samples containing the placebo contaminants (egg white, soybean meal, tofu, and infant formula) (Rodriguez-Saona, Fry, Calvey 2000).

Similarly, the rapid determination of tetracycline in milk was evaluated by FT-NIR spectroscopy (Sivakesava and Irudayaraj 2002b). Tetracycline antibiotics are widely used in animal husbandry for treatment of bacterial infections, suggesting a potential for tetracycline residues to be transferred to milk. The FDA has established a tolerance of
300 ppb for the sum of residues of tetracyclines in milk. It was found that the tetracycline concentration (ppb) range used in calibration models drastically affected the performance of the chemometric models. By using separate ranges the accuracy and predictive ability of the calibration model was significantly improved. Models developed by FT-MIR showed slightly better performance (lower SEP and higher $R^2$) than FT-NIR models but the repeatability of the FT-NIR was better than the FT-MIR procedure (Sivakesava and Irudayaraj 2002b).

Schulz and others (2003) reported the reliable prediction of low concentrations of two carcinogenic compounds: methyleugenol (range 2-235 µg/100g) and estragole (range 34-138 µg/100g) in air-dried basil leaves by PLS calibration model based on NIR spectral data. The performance of the NIR calibration models gave values of SECV of 19.1 and 12.8 µg/100g and coefficient of correlation of 0.95 and 0.89 for methyleugenol and estragole, respectively.

Melamine (2,4,6-triamino-1,3,5-triazine) is used industrially in the production of plastics, glue and also as a plant fertilizer. The compound’s high nitrogen content increases the apparent protein content as measured by traditional protein analysis methods which measure total nitrogen content as an indicator of protein levels. This makes melamine a potential adulterant in protein rich foods such as milk and infant formula. Melamine adulteration has been reported in these products as well as in pet food, candy, coffee drinks, and others. Contaminated milk in China was likely the source of 300,000 cases of renal complications in children and caused at least six deaths (Mauer and others 2009). Currently the FDA uses an LC-MS/MS method to detect melamine in infant formula. The detection limit for this method is 250ppb but it is time consuming and
labor intensive. As a result, it is not efficient for screening large numbers of samples. Detection methods in other food products are also time consuming with varying levels of detection (Mauer and others 2009).

Near- and mid-IR combined with multivariate statistical analysis has enabled researchers to classify adulterated and unadulterated infant formulas with high confidence. FTIR-ATR analysis was done using the regions 3330-2993 cm\(^{-1}\) and 1321-983 cm\(^{-1}\), corresponding to the stretching vibration of amino groups and the fingerprint region, generating a PLSR model with an \(R^2 \geq 0.95\) and RMSECV\(\leq 1\). The NIR model (12497-6098 cm\(^{-1}\) and 5450-4248 cm\(^{-1}\)) performed slightly better based on a unique signal with an \(R^2 = 0.999\) and RMSECV\(=0.62\) (Mauer and others 2009). The FDA has established a threshold of 1 ppm for melamine in infant formula and 2.5 ppm in other foods. Infrared spectroscopy combined with chemometrics has been reported as a rapid method for detecting melamine in milk powder with detection limits of \(~75\text{-}100\ ppm\) (Foss 2009). The technique has shown potential to be used as a tool for screening adulteration in milk with un-specific adulteration detection levels at 250-500 ppm (Foss 2009). Factorization analysis of NIR and MIR spectra was able to distinguish between adulterated (1 ppm) and unadulterated infant formula samples (Mauer and others 2009). NIR and FTIR methods for melamine detection are rapid and the instrumentation is readily available. The limitation of these methods is that they are dependent on the food matrix. New calibration models would be required for different brands of infant formula or for different food products (Mauer and others 2009).

A quality issue was identified by a dairy company regarding the safety of their products. Using a technique very similar to that used for melamine detection, a
methodology was developed for the identification and quantitation of a foreign material found in their milk intake filter. The infrared spectra of the foreign material was collected (Figure 9) and matched with commercial block bait TOM CAT, providing identical IR absorption patterns. Once the foreign material had been identified, the concern shifted to the quality of the milk. The diary company was interested in evaluating milk samples for possible contamination with the foreign material. A PLSR calibration model was developed by spiking uncontaminated milk with known levels of the bait (100 – 2400 ppb). The models had performance statistics of $R^2 > 0.99$ and SECV of ~100 ppb (7 factors) using the infrared from 850 to 1500 cm$^{-1}$, showing potential for the estimation of the bait contaminant levels in few minutes (~2 min). Classification of potential contamination of the milk samples with the bait showed detection limits < 1.2 ppm of the bait (L.E. Rodriguez-Saona, unpublished observations).

![Graph and Image]

**Figure 9:** Attenuated total reflectance (ATR) spectra of the foreign material found in the milk intake filter. Picture of material is shown in insert.
2.5 NEW TECHNOLOGIES

The development of infrared microspectroscopy (IRMS) has allowed for the acquisition of spectra from a sample area measuring only a few microns (Baeten and Dardenne 2002). IRMS combines two analytical technologies for biological analysis by coupling an infinity-corrected microscope to a high performance IR spectrometer equipped with a mercury-cadmium-telluride (MCT) detector that will produce a spectrum with a noise level 10 to 100 times lower than the noise from the commonly used deuterated triglycine sulfate (DTGS) detector. IRMS significantly improves the sensitivity, reproducibility, differentiation and speed capabilities of IR spectroscopy and has permitted the acquisition of spectra from samples as small as 100pg ($10^{-10}$ g), promoting its application in the medical and biological fields (Ozen, Weiss, Mauer 2003). IRMS enables high-throughput screening of chemical contaminants and the ability to resolve spectral profiles within desired regions of the target. The new generation of powerful mid-infrared spectroscopic chemical imaging techniques combines step-scan Fourier-transform Michelson interferometry with indium antimonide focal-plane array (FPA) image detection. The infrared focal-plane array detector provides an instrumental multiplex-multichannel advantage enabling spectra at all pixels to be collected simultaneously, while the interferometer portion of the system allows all the spectral frequencies to be measured concurrently. This high-definition technique represents the future of infrared chemical imaging analysis, which combines the capability of spectroscopy for molecular analysis with the power of visualization. Infrared imaging allows the precise characterization of the chemical composition, domain structure, and
chemical architecture of a variety of substances, information often crucial to understanding of complex samples (Diem 1993).

2.6 CONCLUSION

Vibrational spectroscopic methods such as Fourier-Transform near-infrared (FT-NIR) and Fourier-Transform mid-infrared (FT-MIR) spectroscopy are emerging as powerful techniques in monitoring adulteration and authenticity of foods. In recent years, the food industry and consumers have experienced several new or unsuspected contamination problems such as acrylamide, organic pollutants, Sudan dyes, and recently melamine in dairy products. Analysis of chemical food contaminants and toxins requires the development and validation of analytical methods and their implementation as quality control programs and risk management systems by food producers and authorities. FT-IR spectroscopy is a well-established analytical technique for rapid, high-throughput, non-destructive analysis of a wide range of sample types providing a “fingerprint” characteristic of chemical or biochemical substances present in the sample. Advances in FT-IR instrumentation and multivariate techniques have shown potential for analysis of complex multi-spectral information for the discrimination, classification, quantification and identification of biological systems.

The ability of infrared spectroscopy to reveal qualitative and quantitative characteristics about the nature of chemicals, their structure, interactions and molecular environments provide unparalleled capabilities for detection of contaminants and adulterants in foods. Advantages of approaches based on vibrational spectroscopy include low operational cost, small size, compactness, robustness, high-throughput, ease of use and minimum background training to operate. Thus, FT-IR spectroscopy can provide the
food industry with rapid and specific tools for analysis of food chemical contaminants and for the reliable assessment of quality and safety. It will enable the food manufacturer to rapidly evaluate the quality of their food, allowing for timely correction measures during manufacture.
2.7 REFERENCES


Chapter 3

Application of a Handheld Portable Infrared Sensor to Monitor Oil Quality

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3.1 ABSTRACT

Current methods for quality testing of commercial frying oils are subjective, time consuming, and require hazardous solvents. The objective of this study was to evaluate the capabilities of a portable, handheld infrared spectrometer combined with multivariate analysis to characterize frying oils, to monitor chemical processes occurring during lipid oxidation, and to determine fatty acid composition. This method requires little to no sample preparation, is fast (1-2 minutes) and simple to operate.

Commercial frying oils (corn, peanut, sunflower, safflower, cottonseed and canola) were exposed to accelerated oxidative conditions (65°C) for thirty days. Aliquots were drawn at five day intervals and analyzed by benchtop and handheld mid-infrared devices and reference methods (fatty acid composition (IUPAC 2301, 2302), peroxide values (PV; AOCS Cd 8-53) and free fatty acid (FFA; modified from Shipe 1979). Spectral analysis in the form of Soft Independent Model of Class Analogy (SIMCA) and Partial Least Squares Regression (PLSR) was carried out by pattern recognition software.

Oil samples formed distinct clusters in the SIMCA plots due to difference in their composition. PLSR showed good correlation coefficients (Rval) between reference tests and spectra from the infrared devices. The handheld IR instrument combined with multivariate analysis showed promise for determining oil quality parameters with similar performance as the benchtop units. Its portability and ease-of-use make handheld IR a great alternative to traditional testing methods.
3.2 INTRODUCTION

Producing quality fried snack foods necessitates monitoring the chemical reactions that occur in frying oil during the production process. Lipid oxidation is generally accepted to be a free radical mechanism yielding primary oxidation products (hydroperoxides) which degrade into secondary oxidation products (aldehydes, ketones, etc.) (Farmer, Bloomfield, Sundralingam 1941). This degradation is significant because it has a negative impact on product flavor, shelf life, and nutrition.

Traditional methods for assessing the extent of oxidation measure the concentration of primary or secondary oxidation products. Peroxide value (PV) is one of the most widely used oil testing procedures. As the name implies, it measures the concentration of hydroperoxides in an oil or fat using a titrimetric method and is expressed as milliequivalents active oxygen per kilogram of oil (Guillen and Cabo 2002). Quantifying free fatty acid (FFA) content in oil is a second quality assessment method frequently used by the food industry. FFAs are chemically less stable than triglycerides and therefore are more likely to oxidize and cause rancidity. FFAs are produced during hydrolysis. This reaction is accelerated by high temperatures plus an excess of water-conditions which are common during commercial frying (Lawson 1995). One method for measuring FFA content is based on their conversion to copper soaps, extraction, and reaction with a color reagent. The absorbance is read on a UV-Vis spectrophotometer at 440nm and is plotted on a standard curve (Shipe, Senyk, Fountain 1979). The fatty acid composition of an oil also provides important information about its quality. A fatty acid methyl ester (FAME) procedure is often employed to determine the composition. The
FAME constituents are separated using gas chromatography and the fatty acids are identified by comparison with a reference standard (Indarti and others 2005). These traditional methods are costly, time consuming, and require the use of toxic solvents which then need to be disposed of. Furthermore, they interrupt the flow of manufacturing by requiring that samples be taken into a separate quality laboratory for analysis. As a result, the food industry is seeking alternative methods to ensure the quality of their products.

A potential solution to this problem is infrared spectroscopy. Fourier Transform infrared (FT-IR) spectroscopy combined with multivariate data analysis can provide information on the characteristics, composition, and/or chemical changes occurring in fats and oils. FT-IR methods are generally rapid (1-2 minutes), can be automated, and reduce the need for solvents and toxic reagents associated with wet chemistry methods. In addition, FT-IR can be used for quantitative analysis applications because the intensities of the bands in an absorbance spectrum are proportional to the concentration of their respective functional group (Pare and Belanger 1997). One of the most recent advancements in FT-IR is the development of handheld portable infrared sensors. These are routinely used for chemical identification in the homeland security, public safety, pharmaceutical, and medical markets. The portability of handheld IR spectrometers enables the food manufacturer to bring their quality lab directly to the sample, cutting out a time consuming middle step.

Multivariate statistical analysis techniques are powerful tools that can extract quantitative and qualitative information from FTIR spectra. Developments in the field of multivariate techniques for classification and regression have been prompted by the need
for accurate, robust, and simple methods for routine analysis of spectroscopic and chromatographic data (Udelhoven, Naumann, Schmidt 2000). Spectral data is correlated against a reference method for the constituent of interest, generating calibration models that relate spectral intensities to known analyte concentrations (Thomas and Haaland 1990). Partial least squares regression (PLSR) is a commonly used factor analysis method that has the ability to estimate component concentration and to provide relevant information on chemical and physical properties of the sample from the spectra (Haaland and Thomas 1988). Soft independent modeling by class analogy (SIMCA) is a classification technique based on principal component analysis (PCA) that computes a small number of orthogonal variables that explain as much of the variation as possible between the samples in that class (Martens and Naes 1989; Cowe and McNicol 1985). The principal components preserve the relevant information and eliminate noise.

The aim of this research was to evaluate the capabilities of a handheld portable infrared sensor combined with multivariate analysis to characterize frying oils, to monitor chemical processes occurring during lipid oxidation, and to determine fatty acid composition.

3.3 MATERIALS AND METHODS

3.3.1 Sample Oxidation

Several oil types (refined canola, safflower, and peanut enriched with vitamin E) were purchased from local grocery stores in Columbus, Ohio. Additional oil samples (cottonseed, corn, sunflower, and partially hydrogenated soybean) were supplied by local snack food manufacturers. These oils represent the types of oils traditionally used for frying in the United States (Matthaus 2007).
To prepare for oxidation, four hundred milliliters of each oil sample was measured into 500mL Erlenmeyer flasks. The oxidation of each sample was carried out in duplicate. Uncovered flasks were heated to 65°C (±1) in an Isotemp incubator (Fischer Scientific) to accelerate oxidative conditions. Fifteen milliliter aliquots of each sample were drawn every five days for 30 days to be analyzed using reference methods and for FT-IR spectra collection. The aliquots were stored in glass vials at -14°C until analysis.

3.3.2 Peroxide Value and Free Fatty Acid Reference Tests

Peroxide value (PV) was measured using the AOCS official method (Cd 8-53 Peroxide values of oils and fats). Five grams (± 0.05 g) of oil was weighed into a 250 mL Erlenmeyer flask and 30 mL 3:2 acetic acid-chloroform solution was added. The solution was mixed until the sample dissolved and 0.5 mL of a saturated potassium iodine solution was added. After one minute, thirty milliliters of distilled water was added to the flask. This mixture was titrated with 0.01N sodium thiosulfate while continuously swirling until the color changed to light yellow. At this point, a 10% soluble starch indicator solution was added (1 mL) to give a blue color. The titration was continued until all of the iodine was liberated from the chloroform layer, with the end point being the transition from blue to colorless.

The method to determine the free fatty acids in oil was derived from Shipe et al (1979). The reagents were prepared first. For a copper soap reagent, 5 mL triethanolamine was added to 10 mL 1 M Cu(NO₃)₂. This solution was diluted with 100 mL saturated NaCl solution. The pH was adjusted to 8.3 with 1N NaOH. A 0.5% sodium diethylcarbonate color reagent was prepared using n-butanol as the solvent.
To construct a standard curve, a 0.04% solution of palmitic acid in 49:49:2 chloroform:heptane:methanol (CHM) was prepared. Into polypropylene test tubes, varying amounts of the 0.04% palmitic acid solution were added, ranging from zero to three milliliters. The tubes were filled to 5 mL with CHM. Distilled water (0.5 mL) and 0.1 mL of 0.7N HCl was added to the test tubes and shaken vertically for 30 minutes using a Babcock shaker set to 470 rpm. Two milliliters of copper soap reagent was added to the test tubes and they were shaken for 10 minutes. Three milliliters of the clear supernatant was transferred into a glass test tube (13 x 100 mm disposable borosilicate glass culture test tube) containing 0.5 mL color reagent and the absorbance was immediately read on a spectrophotometer set to 440 nm. The spectrophotometer was zeroed with the prepared solution containing no palmitic acid.

To test the oil samples, 0.1 mL of 0.7 N HCl was added to 0.5 mL of oil sample in a polypropylene tube. In order to prevent an emulsion, 0.1 mL of 1% Triton X 100 solution was added to the test tubes and vortexed. Two milliliters of copper soap reagent was added and the test tubes were again vortexed. Six milliliters of CHM solvent was added to the test tubes and the test tubes were mixed for 30 minutes on a Babcock shaker at 470 rpm. The supernatant (3.5 mL) was transferred into a test tube (13 x 100 mm disposable borosilicate glass culture test tube) with 0.1 mL color reagent. The samples were done one at a time. As soon as the color reagent was added, the sample was read on the spectrophotometer. The amount of palmitic acid in micrograms is extrapolated from the standard curve. The milliequivalent conversion represents the entire free fatty acid content in the sample, not just palmitic acid.
3.3.3 Fatty Acid Composition

The fatty acid composition of the oils was determined using a fatty acid methyl ester (FAME) procedure (Rodriguez-Saona, Barrett, Selivonchick 1995). In a method modified from Rodriguez-Saona (1995), esterification was achieved by adding 10 mL of 4% methanolic-sulfuric acid to 0.05 mL oil sample plus 1mL benzene in a glass test tube with a Teflon screw top cap. The mixture was heated to 80-90°C for 90 minutes. Methyl esters were extracted using a partition of hexane and distilled water. One milliliter of the hexane portion was collected in a 1.5 mL GC vial with crimp top and was evaporated under nitrogen. The dried samples were re-diluted using 0.5 mL iso-octane. The samples were analyzed on an HP-6890 GC equipped with a flame ionization detector (FID). An HP G 1513A autosampler and tray were used to automate the injections. Separation of the components was done using an HP-FFAP 25 m x 0.32 mm x 0.5 um column (Agilent part number 19019F-112) using helium as the carrier gas. The injection volume was 1 µL with a split ratio of 20:1. The oven conditions were 110°C (1 min), to 220°C (5°C/min) hold for 15 minutes. The injector temperature was 220°C and the detector temperature was 250°C. The identification of fatty acids was carried out by comparing the retention times with a reference standard (NuChek Prep GC standard 15A).

3.3.4 Spectra Collection

Infrared spectral data was collected on several instruments. Oil samples (50 µL) were analyzed on a single bounce FatIR with a ZnSe crystal attached to a temperature controller (Harrick, Pleasantville, NY) maintained at 65°C. A Varian Excalibur 3100 spectrometer (Varian, Palo Alto, CA) was used with a KBr beam splitter and Deuterated Triglycine Sulfate (DTGS) detector, operating at 4 cm⁻¹ resolution. Spectra were
collected over the frequency range of 4000-700 cm\(^{-1}\) and interferogram of 64 scans were co-added according to Beer-Norton apodization. Spectra were displayed in terms of absorbance and viewed using Win-IR Pro Software (Varian, Palo Alto, California). Each sample was analyzed in duplicate. To prevent interference in the spectra, the instrument was continuously purged with CO\(_2\) from a CO\(_2\)RP140 dryer (Dominic Hunter, Charlotte, NC, USA). Samples were also analyzed using a triple bounce ATR ZnSe crystal (Pike Technologies) on a Varian Excalibur 3100 Spectrometer (Varian, Palo Alto, CA). The same beam splitter and detector used for the FatIR were used for these readings at a resolution of 8 cm\(^{-1}\). Spectra were collected over the frequency range of 4000-700 cm\(^{-1}\) and interferogram of 128 scans were co-added. Samples were maintained at 65°C prior to analysis. A handheld FTIR spectrometer was used in addition to the benchtop equipment. The handheld unit had a spectral range of 4000-650 cm\(^{-1}\) and a resolution of 16 cm\(^{-1}\) with an ATR diamond crystal. The samples (40 µL) were heated to 65°C before analysis.

### 3.3.5 Data Analysis

All spectral data was exported as GRAMS.spc file format and were imported into Pirouette for Windows Comprehensive Chemometrics Modeling Software, version 3.11 (Infometrix, Inc. Bothell, WA). The spectra were analyzed by soft independent modeling of class analogy (SIMCA) to generate classes. The multivariate analysis program allows for visualization of clustering among samples. SIMCA was used to evaluate the ability of the spectra to discriminate oils based on their composition and degree of oxidation. After training sets assigned data to classes, principle component models were generated. The identities of unknown samples were predicted by where they fell in relation to the classes
in the training sets. The unknown sample could be part of one class, part of more than one class, or not part of a class.

Pirouette software was also used for partial least squares regression (PLSR) to model the relationship between two factors in a linear fashion. These models estimated the peroxide value or free fatty acid level from the spectra using a cross-validation, leave one out approach. The standard error of calibration (SEC), standard error of cross-validation (SECV), and the coefficient of correlation (Rval) were used to evaluate the validity of the PLSR models.

3.4 RESULTS

3.4.1 Peroxide Value

The peroxide value of all oil types (peanut, safflower, canola, sunflower, corn, and cottonseed) measured by iodometric titration followed a general pattern of increasing over the thirty day study, indicating that oxidation was proceeding (Figure 10). This trend matches that observed in previous rancidity studies (Jarvi and others 1971; Schnepf, Spencer, Carlat 1991). All oils had an initial PV close to zero. Cottonseed and corn oils had the highest values at the end of the study (Figure 10). This is likely due to the high levels of unsaturation in these oils. Corn oil is composed of approximately 87% unsaturated fatty acids and cottonseed oil is approximately 74% unsaturated (Eyres 1995). Mistry and Min (1992) found that as unsaturation increases, the rate of oxidation also increases. This does not explain why the canola oil sample had the lowest PV at the end of the study. Canola oil has the highest level of linolenic acid (10%) out of the oils studied and therefore should have the fastest rate of oxidation (Eyres 1995). It is possible that an antioxidant was added to the oil by the manufacturer, slowing down the rate of oxidation.
oxidation. The oil samples reached a PV of 10 around day 5 (Figure 10). This value is significant because a PV over 10 is commonly associated with off odor and flavor in oils (Allen and Hamilton 1994).

![Figure 10: Average peroxide value (PV) of 6 oil types over a 30 day stability study in which samples were held at 65°C to accelerate oxidation. PV determined using AOCS method Cd 8-53.]

### 3.4.2 Free Fatty Acid

Free fatty acid levels of the oils increased over thirty days as has been previously observed (Figure 11). Free fatty acids are less stable and are susceptible to oxidation, while hydroperoxide free radicals serve as oxidizing agents. Peanut, canola, corn and sunflower oils (~0.5%) had similar initial free fatty acid levels while peanut oil (0.8%) and safflower (1.0%) showed higher acid values. Sunflower oil showed the highest hydrolytic stability taking 25 days to reach an acid value of 1% while canola oil showed the highest hydrolytic rancidity among oils, reaching an PV of 4.3% after 30 days of
treatment. Further, the samples would have hydrolyzed even faster if they were used for frying snack foods due to the increase in moisture. The rates of oxidative and hydrolytic rancidity for the different oils are not identical because of the differing mechanisms (Mistry and Min 1992, Guillen and Cabo 1997). Oxidative rancidity is strongly dependent on the degree of saturation and highly unsaturated fatty acids are the most susceptible to being oxidized (Mistry and Min 1992).

Figure 11: Average free fatty acid (FFA) content of 6 oil types over a 30 day stability study in which samples were held at 65°C to accelerate oxidation. FFA determined using a method derived from Shipe et al (1979).
3.4.3 Fatty Acid Composition

The major fatty acids (Figure 12) present in oils were identified as stearic, palmitic, oleic, linoleic, and linolenic acids and were grouped into saturated (stearic and palmitic), monounsaturated (oleic) and polyunsaturated (linoleic and linolenic) fatty acids. Corn and cottonseed oils showed the highest levels of polyunsaturated fatty acids (PUFA) (>3%) while the rest of oils showed PUFA levels ~1.5%. Canola (4.6%) and safflower (6.1%) showed the highest levels of monounsaturated oil. There was no statistically significant difference (p>0.1) in the levels of fatty acids in oils during the 30-day study. Higher monounsaturated fatty acids and low PUFAs levels were related to improved oxidative stability of frying oils. Figure 13 shows a sample chromatogram from the oils tested. Good resolution was achieved and peaks were symmetric.

Figure 12: Standard curve of methyl oleate quantifying sample amount based on peak GC chromatogram peak area (left). Table of methyl ester elution times and correlation coefficients ($R^2$) of standard curves (right)
Figure 13: Chromatogram of fresh corn oil collected on a gas chromatograph with a flame ionizing detector

3.4.4 Spectra Collection

Aliquots of the oil samples (40-50 µl) were placed directly on the infrared crystals. A scan time of one to two minutes and no sample preparation makes infrared an incredibly fast and efficient method for edible oils. The FTIR spectra reflect the functional group composition of the samples and the band intensities vary with the overall concentration of the functional groups. All the oils provided well-defined and consistent spectra and ATR helped to maintain a fixed path length for infrared light interaction, thereby eliminating the need for internal standards. Heating the ATR crystal helped maintain the physical state of the oils (liquid) and improved contact of the oil with the surface. The raw spectra were transformed into their second derivatives to remove the baseline shifts, improve band resolution, and reduce noise and variability between replicates (Kansiz and others 1999). Similar infrared profiles were obtained by all spectrometers (handheld and benchtops)(Figure 14). Band assignments in the oil
spectrum have been summarized by several authors (Vlachos and others 2006; Guillén and Cabo 1997). The most prominent absorption regions were the -C=CH cis, CH₂, and CH₃ stretching vibrations between 3050-2800 cm⁻¹, stretching vibrations of double bonds (-C=O of esters) between 1750 and 1650 cm⁻¹, bending and rocking vibrations of CH, CH₂, and CH₃ groups between 1465 and 1370 cm⁻¹, and the -C-O stretching and CH₂ rocking vibrations in the fingerprint region (1250 to 700 cm⁻¹). Hydroperoxides (O-H) absorbs between 3470 and 3445 cm⁻¹ and ester bond of free fatty acids absorb at 1711 cm⁻¹.

![Figure 14: Comparison of safflower oil mid infrared spectra (4000-700 cm⁻¹) produced by infrared instruments- a portable handheld FTIR, a benchtop MidIR with FatIR accessory, and a benchtop MidIR with triple bounce ATR accessory](image)

**3.4.5 Data Analysis**

The collected spectra of oils were analyzed by SIMCA in order to determine the spectral differences between them. The SIMCA procedure is a principal component analysis (PCA) technique that works by reducing the dimension of multivariate data sets. In SIMCA, known spectra (training sets) are assigned to specific classes and a principal component (PC) model is generated for each class with distinct confidence regions within
them (Maesschalck and others 1999). All six samples formed distinct well separated clusters in the three dimensional space formed by the three PC axes. The greater the distance between the clusters the greater is the difference in their chemical composition. This multivariate approach has been previously reported to classify oils based on type using a full resolution benchtop MidIR (van de Voort and others 1994). SIMCA was able to use the handheld data to classify the oils, generating distinct clusters (Figure 15). Whether the generated classes were statistically different was determined using interclass distances (ICD) and distances of >3 are considered significant (Dunn and Wold 1995). Nearly all of the ICD for the handheld data were greater than or equal to three (Table 5), indicating excellent separation of the oil types. SIMCA classes generated using spectra from the benchtop MidIR with triple bounce ATR showed tightly clustered and well separated groups (Figure 2). The ICD from the infrared benchtop models were the highest ranging from 15 to 96, indicating improved spectral resolution over the handheld unit (Table 6). The chemical differences responsible for the separation of oil classes in the SIMCA plot were identified using the discriminating power plot. In the discriminating power plot each wavenumber in the spectral range is plotted against its power in discriminating the samples that are being compared. The higher the value of discriminating power, the greater is difference between the samples in functional groups or bonds associated with that wavenumber. The discriminating power plot and the important spectral wavenumbers responsible for the classifications of the oils on Day 0 (fresh) are shown in Figure 17. The major differences between the oils were in the infrared bands around 3012 cm\(^{-1}\) and 2920 cm\(^{-1}\), which correspond to \(=C-H\) (cis-).
stretching and –C-H (CH$_2$) asymmetric stretching, respectively. In terms of fatty acid composition, these correspond to differences in double bonds or the level of unsaturation and the length of carbon chains. While the benchtop showed improved spectral resolution compared to the handheld unit, the classification models relied on the same region of the spectra for explaining variance within the data (Figure 17).

After 30 days of stressing the oils at 65°C, the oils showed significant spectral differences from the fresh oil (Figure 18). Differences in chemical structures responsible for discrimination were different from those of fresh oils attributed to thermal stress (data not shown). The importance of the band at 3012 cm$^{-1}$ was not as pronounced as in fresh oils. The main discriminating band in the treated oils was 1740 cm$^{-1}$, associated with stretching vibrations of the ester (–C=O) linkage. Changes in the chain length due to oxidation of fatty acids at the double bonds was also evident from the increase in discriminating power at 2920 (-C-H$_2$ asymmetric stretching) and 2850 cm$^{-1}$ (-C-H$_2$ symmetric stretching).

![Figure 15: SIMCA classification plot for discrimination of oil types using spectra from a handheld portable FTIR](image)

Figure 15: SIMCA classification plot for discrimination of oil types using spectra from a handheld portable FTIR
**Table 5**: SIMCA interclass distances among 6 oil types using spectra from a handheld portable FTIR

<table>
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<tr>
<th></th>
<th>canola</th>
<th>peanut</th>
<th>safflower</th>
<th>corn</th>
<th>cottonseed</th>
<th>sunflower</th>
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<td>11.45</td>
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<td>sunflower</td>
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<td>2.73</td>
<td>2.20</td>
<td>13.13</td>
<td>5.84</td>
<td>0</td>
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</tbody>
</table>

**Figure 16**: SIMCA classification plot for discrimination of oil types using benchtop MidIR with triple bounce ATR

**Table 6**: SIMCA interclass distances among 6 oil types using spectra from a benchtop MidIR with triple bounce ATR

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<thead>
<tr>
<th></th>
<th>canola</th>
<th>peanut</th>
<th>safflower</th>
<th>corn</th>
<th>cottonseed</th>
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<td></td>
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<td>sunflower</td>
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<td>28.88</td>
<td>29.26</td>
<td>68.74</td>
<td>53.78</td>
<td>0</td>
</tr>
</tbody>
</table>
Partial least squares regression was used to correlate oxidative reference tests with spectral data from the handheld and benchtop MidIR instruments (Figure 19). PLS, a bilinear regression analysis, determines the concentration of a dependent variable, X (concentration of analyte) by regressing a small number of orthogonal factors (also known as latent variables) that are linear combinations of independent variables, Y (infrared wavenumbers). These latent variables explain as much covariance as possible between the dependent and independent variables (Bjorsvik and Martens 1992). PLSR
models using spectra of all vegetable oils collected by the handheld FTIR gave coefficients of correlation (Rval) of 0.84 and 0.93 for FFA and PV, respectively (Table 7). The SECV value for the handheld unit was 0.55% for FFA and 9.5 meq/Kg for PV. Overall, the handheld generated weaker calibration models with twice the error compared to the benchtop instruments (Table 7). Improved statistics were obtained for PLS calibration models developed for individual oils to predict FFA and PV (data not shown). Our findings clearly demonstrate the ability of mid-infrared spectroscopy combined with multivariate PLS analysis to predict FFA and PV and fatty acids independent of the oil type.

![Figure 18: PLSR model of peroxide value (PV) reference tests of peanut, canola and safflower oil from the handheld FTIR (left) and benchtop with ATR (right).](image)

**Table 7:** Summary of statistical analyses of PV and FFA from all infrared sources using the region 900-1800 and 2700-3200 cm\(^{-1}\)

<table>
<thead>
<tr>
<th>Technique</th>
<th>Factors</th>
<th>SECV(^a)</th>
<th>Rval(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MidIR with ATR</td>
<td>8</td>
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<td>0.94</td>
</tr>
<tr>
<td>FatIR</td>
<td>9</td>
<td>0.33</td>
<td>0.95</td>
</tr>
<tr>
<td>TruDefenderFT</td>
<td>5</td>
<td>0.55</td>
<td>0.84</td>
</tr>
<tr>
<td>PV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MidIR with ATR</td>
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<td>4.22</td>
<td>0.99</td>
</tr>
<tr>
<td>FatIR</td>
<td>9</td>
<td>6.48</td>
<td>0.97</td>
</tr>
<tr>
<td>TruDefenderFT</td>
<td>9</td>
<td>9.57</td>
<td>0.93</td>
</tr>
</tbody>
</table>

\(^a\)standard error of cross validation. \(^b\)coefficient of correlation for validation model.
The final objective of this study was to use handheld spectral data combined with multivariate statistical analysis to determine fatty acid composition. Spectra from the handheld unit were correlated with quantities of saturated, monounsaturated, and polyunsaturated fatty acids that had been determined using the FAME method. Strong correlations were displayed between the spectra of a set of three different oils (corn, cotton, and sunflower) and the different levels of saturation (Table 8). The same models were generated using spectra from the benchtop MidIR with triple bounce ATR. The handheld and benchtop PLSR models for monounsaturated fat were nearly identical. Both models required four factors to explain the variance in the data and had standard errors of 0.31 and 0.30 respectively and coefficient of correlation >0.94. The PLSR model of polyunsaturated fat was the handheld’s most robust model with an $R^2=0.96$ (Figure 20).

Figure 20: PLSR model of polyunsaturated fatty acid composition of sunflower, cottonseed, and corn oil from a handheld FTIR (left) and benchtop with ATR (right)
3.5 CONCLUSION

The results of this experiment show that a handheld portable FTIR is a valuable device for characterizing frying oils, monitoring chemical processes occurring during lipid oxidation, and for determining fatty acid composition when combined with multivariate statistical analysis. The benefits of being a portable device that is easy to learn and operate outweigh the limitations of reduced sensitivity.

Currently these units come equipped with only a basic internal library. As a result, spectra have to be exported into a computer in order for the data to be analyzed by a chemometric program such as Pirouette. Integration of an advanced chemometrics program into the device would save the user even more time and would further simplify the process. It is also important to note that statistical models are not easily transferred from one infrared device to another. This means that new models have to be created for each handheld device. Fortunately, creating the models is a fairly simple process.

It is anticipated that handheld FTIR units could be used to ensure the correct oil type is being used in a process and that it is free of adulterants. It could also be used to simplify the process of identifying fatty acid composition for labeling purposes. Most importantly, the handheld technology enables the food manufacturer to bring the quality of their products to a higher standard.

| Table 8: Summary of statistical analysis for determining fatty acid composition |
|------------------------------|--------|-----------|----------|
|                              | Technique        | Factors | SECV\(^a\) | r\(^{val}\)^b |
| saturated fat                | MidIR with ATR   | 6       | 0.08      | 0.97       |
|                              | TruDefenderFT    | 4       | 0.12      | 0.95       |
| mono-unsaturated fat         | MidIR with ATR   | 4       | 0.31      | 0.94       |
|                              | TruDefenderFT    | 4       | 0.3       | 0.95       |
| poly-unsaturated fat         | MidIR with ATR   | 8       | 0.16      | 0.98       |
|                              | TruDefenderFT    | 6       | 0.24      | 0.96       |

\(^a\)standard error of cross validation. \(^b\)coefficient of correlation for validation model.
lab to the sample in order to monitor lipid oxidation. Little training is needed in order to operate the infrared unit meaning that it can be operated by individuals other than QC personnel. This process would save manufacturers time, money, and other valuable resources.
3.6 REFERENCES


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