Application of Mid-Infrared Spectrometers in Determination and Quantification of

Trans-fatty Acid Content in Snack Foods and Bakery Products

THESIS

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Alex Milligan

Graduate Program in Food Science and Technology

The Ohio State University

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Master's Examination Committee:

Dr. Luis Rodriguez-Saona, Advisor

Dr. Ahmed Yousef

Dr. Lynn Knipe
Abstract

Nutritional labels often under report trans-fat content. Due to the health problems associated with consumption of trans-fats, efforts must be made to ensure careful monitoring and enforcement of current guidelines. Recent FDA press releases indicate that trans-fat will be removed from the GRAS substances list in the near future. If such regulation were to be enacted, it would effectively act as a ban on all trans-fats in food. The objective of this study was to isolate and quantify trans-fat content in a variety of local food products reporting some level of trans-fat in the product and approximate the prevalence of misrepresentation of trans-fat levels across several types of foods. Isolation of trans-fatty acids from locally obtained food products was achieved using AOAC Official Method 2000.10 and analysis was performed using the Cary 630 portable FTIR. A standard curve was constructed using trielaidin at varying concentrations. Isolated trans-fats from 40 food products were analyzed; three replicates were run for each product. Spectral data examined using partial least squares regression (PLSR) showed very good correlations ($R^2 > 0.998$) for models produced using both spectrometers. Portable ATR-MIR spectrometers allow for increased flexibility in set up and use while retaining the traditional benefits of FTIR spectroscopy such as rapid throughput, high sensitivity, and large amounts of data per second, making it ideal for regulatory applications and well suited to quality control applications.
Acknowledgments

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Vita

2007......................................................Hopkinsville High School

2011..........................................................B.S. Biology, University of Kentucky

2012 to present ........................................Graduate Teaching Associate, Department

of Food Science, The Ohio State University

Fields of Study

Major Field: Food Science and Technology
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Chapter 1: Literature Review

1.1 Trans Fat

The American Heart Association recommends consuming between 25 to 35 percent of your daily calories as fats (American Heart Association, 2010). This amounts to approximately 65g of fat and oil per day, and due to the great variety of available fats in addition to the negative stigma associated with fatty foods, deciding which fat to include in meals and snacks has been a major source of anxiety for consumers, the food industry, and researchers for much of the last century (American Heart Association, 2010). Until the advent of large scale agriculture and advanced processing techniques, dietary fat primarily consisted of butterfat, beef tallow, and lard (Klonoff 2007). When demand for butter eclipsed the supply capacity, manufacturers of the mid 1900s began to apply the process of hydrogenation to produce inexpensive saturated fats (Remig et al. 2010).

Due to great overabundance resulting from soybean production as a protein source, soybean oil was one of the primary initial sources of partially hydrogenated oil in the American diet and continues to be one of the largest contributors (Kummerow 2008; Vega-Lopez et al. 2006; Eckel et al. 2007). Soybean oil could be acquired cheaply and then be modified using heat, pressure, hydrogen gas, and metal catalysts in order to
confer properties similar to that of butter for use in baked goods (Fennema 2008). To promote desirable storage and baking characteristics, manufacturers target a degree of saturation that gives a fat that is solid at room temperature, but melts at baking temperatures. The resulting partially hydrogenated oils have greatly increased shelf life and flavor stability and are inexpensive when compared to butter and other animal fats (Remig et al. 2010). Early proponents of these partially hydrogenated oils (PHOs) touted the health benefits of consuming more unsaturated fats as opposed to the saturated fats found in animal fats. This assertion was difficult to refute until the 1990s as studies up to that point were inconclusive with regard to the effects on trans-fat consumption on human health (Eckel et al. 2007).

The first study to confirm the effect of trans-fats on the ratio of good cholesterol to bad cholesterol was published in 1990 (Mensink & Katan 1990). This study compared diets rich in monounsaturated oils, saturated fats, and trans-fats and found the trans-fat rich diet to have the greatest negative effect on this ratio. Research of the modern era continues to unanimously agree that trans-fats resulting from PHO production are a leading cause of Coronary Heart Disease (CHD) over the last century (Ascherio et al. 2006; Hu et al. 2001); one such study found that a 2% increase in energy intake from trans-fat increased the risk of CHD by greater than 20% (Mozaffarian et al. 2006).

In 1993, after publishing an article in The Lancet linking trans-fat consumption with CHD in women (Willet et al. 1993) the Harvard School of Public Health (HSPH) began
advocating for the required labeling of trans-fats due to the public health hazard they presented. As a result of HSPH and other group’s findings, in November 1999, the Food and Drug Administration (FDA) announced that they planned to include trans-fatty acid content on the standard food label. It wasn’t until January 1, 2006, that FDA guidelines requiring the labeling of trans-fat in foods took effect (Food and Drug Administration, 2003). It is important to note that according to Code of Federal Regulations (CFR) 21, any food containing less than 0.5 g of fat per serving may be considered fat free (Food and Drug Administration, 1978). This regulation encompasses trans-fatty acids in the same way and as such, foods containing partially hydrogenated oils may legally claim 0 g trans-fat on their label while containing as much as 0.49 g per serving of trans-fatty acids. Additionally, there is a 20% allowance for batch variance such that the 0.5g per serving allowance expands to 0.6g per serving (Food and Drug Administration, 1978).

Fast forwarding to more recent times, the FDA has begun to take steps to eliminate PHOs from the food supply all together. In November of 2013, the FDA released an announcement proposing that Partially Hydrogenated Oil lose their current GRAS (Generally Recognized As Safe) status (Food and Drug Administration, 2013). Since this initial release, there have been several updates relaying the FDA’s intention to extend the comment period before official action is taken. These extensions are to be expected and do not mitigate the likelihood that all food producers will have to apply for pre-market approval to the FDA for the use of trans-fat in their foods in the near future and in so doing, they must provide data to prove that PHOs can be reasonably expected to cause no
harm to human health. Because this will be all but impossible to prove, loss of GRAS status would essentially act as a ban on PHOs. This proposed regulation does not include \textit{trans}-fatty acids (TFA) from natural sources such as the \textit{vaccinic acid} and \textit{conjugated linoleic acid} (CLA) found in the meat and milk of ruminant feed animals (Food and Drug Administration, 2013; Remig et al. 2010). This distinction further highlights the importance of accurate detection and determination of TFA content going forward. If the food supply is to be kept free of PHOs, accurate and rapid detection methods must continue to be developed and vetted.

The Food and Drug Administration does not view \textit{trans}-fat from natural sources in the negative light by which they view PHOs due to a lack of credible research linking natural \textit{trans}-fats to the health problems brought about by PHOs. One reason for this biological distinction is that \textit{trans}-fats from natural sources have lower melting points than PHOs, which are notably solid at body temperature (Fennema, 2008). The much higher melting point of PHOs is a result of the long linear fatty acids ability to pack together very tightly (Kummerow 2008; Fennema, 2008), natural \textit{trans}-fats are more sterically complex and, as such, do not associate as tightly (Remig et al. 2010). The long, linear configuration of PHOs leads to the production of larger micelles, the fat-conducing lipoproteins found in the bloodstream. These larger micelles tend to be of lower density and are thus more inclined to be found to the edges of blood vessels due to the physics of laminar flow (Hu et al. 2001). Once near the arterial walls, some of these low density lipoproteins, LDLs, are deposited on the inside of the arteries and blood vessels leading to plaque formations.
and lessened effective width within arteries (Hu et al. 2001). Additionally concerning, aside from increasing the production of LDLs, *trans*-fat consumption has been found to decrease the production of high density lipoproteins, or HDLs, which tend to reverse some of the negative effects of LDLs (Ascherio et al. 2006). All of this results in increased arterial pressure and other problems such as coronary heart disease (Almendingen et al. 1995). In addition to increasing risk of cardiovascular diseases, PHO consumption has been linked to infertility, endometriosis, gallstones, Alzheimer’s disease, diabetes and some cancers (Almendingen et al. 1996; Teegala et al. 2009). Natural sources of *trans*-fat have not been shown to cause this propensity for higher percentages of LDLs in the body, and have been linked to other beneficial effects such as cancer prevention and lowering cholesterol (Ascherio & Willett 1997).

That consumption of *trans*-fats results in a variety of health problems is a long held truth. There have been attempts to limit *trans*-fat consumption throughout the nation and world with varying degrees of success. At least 26 countries have *trans*-fat regulations in effect, but most only aim to limit final products’ PHO content (Stender et al. 2006). Denmark has had success with its attempts to limit the intake of industrial produced *trans*-fatty acids (IP-TFA) by strictly regulating the maximum amount of IP-TFA that can be found in fats and oils destined for human consumption to 2% *trans* (Eckel et al. 2007). This ingredients focused approach has been adopted by several other countries including Switzerland and Sweden (Eckel et al. 2007; Stender et al. 2006).
Within the United States the best known examples of regulatory limitations on trans-fats originate from the state of California and New York City’s campaigns. In California, 2008 legislation placed limits on the use of trans-fat in restaurants and food-preparation facilities with very good success, more or less eliminating trans-fats in the state from all but packaged food products (Gorn 2013). In NYC, the story is more convoluted. The process began in 2005 with a public education program and a request to restaurant owners to eliminate trans-fat from their offerings (Mello 2009). After determining that restaurant owners were not complying with this request, the city’s Board of Health voted to ban trans-fat in restaurant food on December 5, 2006 (Mello 2009). Despite loud criticism at the time, the ban has been a great success according to the NYC’s Board of Health and other national health agencies (Valentina et al. 2010). Companies both large and small were forced to reformulate their products to preclude trans-fat; there were very few reported cases of consumers noticing any changes (Mello 2009). That these companies were able to reformulate without noticeable quality loss undoubtedly empowered the FDA to move ahead in its current engagement with PHOs. The successes in California, New York, and abroad have shown us that the choice of fat to replace IP-TFA depends upon the product in question and will change based on availability and desired quality characteristics. That said, the replacement fat will almost assuredly cost more and reduce product’s shelf lives, at least in the immediate future (Stender et al. 2006).

The first step in beginning to understand what fat ought to be used to replaced trans-fat in a given food product is to know whether the trans-fat was used as a frying oil or solid fat.
The health effects associated with \textit{trans}-fats in addition to the fact that they are tasteless exclude them from use in cooking or salad oils (Remig et al. 2010). In product developers’ quest to retain quality and shelf life while eliminating PHOs, foods in which the \textit{trans}-fat is used as a solid fat will be the toughest to replace. In these products, the functionality conferred by TFAs really shines and, as such, will be tougher reformulations. Some such products include: baked good, refrigerated dough products, coffee creamers, canned frosting, and frozen goods. In these products TFAs provide a thick, buttery mouthfeel and an extended shelf life with little quality degradation. From a quality standpoint TFAs bind to the structural matrix of foods more strongly than other fats in addition to being highly resistant to oxidation (Valentina et al. 2010). Identifying replacement fats with these qualities will take time, but in the interim it is most likely that novel mixtures of several vegetable oils becomes the standard with variation to occur primarily in saturated fat content with little or no change to total fat content (Eckel et al. 2007). There are many options out there, and with the vacancy in the market that PHOs will leave behind there will undoubtedly be plenty of effort given to producing better options, but care must be taken to avoid adopting a fat that is even more detrimental to human health.

In 2006 the American Heart Association convened the \textit{Trans} Fat Conference in Washington, DC. The primary purpose was to discuss the status of the reduction of \textit{trans}-fatty acids in the American diet and the future implications of doing so (Eckel et al. 2007). They decided that when reformulating to preclude \textit{trans}-fats, the most important considerations are: availability, health effects, consumer acceptance, research and
development investments, reformulated food quality and taste, supply-chain management, operational modifications, and cost (Eckel et al. 2007). An overview of the strategies that were discussed during this conference is shown below (Eckel et al. 2007).

**Table 1. Overview of strategies for reduction of trans-fat in the American diet.** (adapted from: Table 5. Alternatives to Partially Hydrogenated Fat. Eckel, Borra, Lichtenstein, & Yin-Piazza 2007)

<table>
<thead>
<tr>
<th>Alternative</th>
<th>Description</th>
<th>Example</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropical oils</td>
<td>Oils that come from plants in the tropics</td>
<td>Palm oil, Palm kernel oil, Coconut oil</td>
<td>Functionality, Economics, Availability, Consumer acceptance</td>
<td>Negative health effect associated with high saturated fat content</td>
</tr>
<tr>
<td>Animal fats and edible tallow</td>
<td>Fats that come from animals</td>
<td>Beef tallow, Lard, Butter</td>
<td>Functionality, Consumer acceptance</td>
<td>Negative health effect associated with high saturated fat content and naturally occurring cholesterol</td>
</tr>
<tr>
<td>Trait-enhanced oils</td>
<td>New oilseed varieties that can yield oils that are stable without requiring hydrogenation. Most developed by the oilseed industry, sometimes in collaboration with the USDA using conventional plant breeding</td>
<td>Low-linoleic soybean and coi oils. Mid-oleic soybean and sunflower oils. High-oleic soybean, sunflower, and canola oils.</td>
<td>Many new varieties have been developed or are in research and development pipeline. Generally acceptable functionality for frying.</td>
<td>Generally higher costs. Long lead time for delivery. Uncertainties regarding availability.</td>
</tr>
<tr>
<td>Blending liquid soft oils with harder components</td>
<td>Blending partially hydrogenated vegetable oils, fully hydrogenated vegetable oils (with polyunsaturated fatty acid and monounsaturated fatty acid converted to stearic acid), or tropical fats with liquid vegetable oils</td>
<td>Company-specific products</td>
<td>Individually formulated to provide various fatty acid compositions and melting profiles. Used for frying or baking depending on the fluidity of the fat.</td>
<td></td>
</tr>
<tr>
<td>Modified hydrogenation process</td>
<td>Increasing the pressure, decreasing the temperature, and/or changing the catalyst or catalyst concentration to lower levels of trans-fatty acids.</td>
<td>Company-specific products</td>
<td>Can selectively reduce the amount of trans-fatty acids produced during hydrogenation. In some cases, trans-fatty acid production has been suppressed by up to 80%.</td>
<td>Extremely high pressure and concentrations of catalysts required can reduce commercial viability.</td>
</tr>
<tr>
<td>Fractionation of tropical fats</td>
<td>Separating palm oil into hard fractions to be used as</td>
<td>Company-specific products</td>
<td>Fractions with different solid fat profiles and melting point curves to allow versatility in formulation</td>
<td>Negative health effect associated with highly saturated hard fractions (including palmitic (C16:0) and palm kernel oil containing C12, C14, and C16). Process highly developed in Europe but not in the US.</td>
</tr>
<tr>
<td>Interesterification</td>
<td>A liquid and a hard stock (e.g. palm kernel oil, solid palm fraction) are blended together and interesterified. Involves treating a fat with an excess of glycerol in the presence of a chemical or an enzymatic catalyst at a relatively low temperature, causing the rearrangement or redistribution of the fatty acids on the glycerol portion of the molecule, thus producing fat with different melting profiles and physical characteristics than the parent.</td>
<td>Company-specific products</td>
<td>Does not change the degree of unsaturation of the fatty acids. Does not convert cis into trans isomers. If an enzymatic catalyst is used, resulting interesterification process is continuous and specific, with steeper solid fat curves to provide better functionality and few unidentified byproducts with the need for extensive post processing.</td>
<td>High cost of the enzymatic catalyst. Technology has been fully examined for its effects on health.</td>
</tr>
</tbody>
</table>
When looking through Table 1, it is noteworthy that a major goal of the conference was to transition away from trans-fatty acids without increased consumption of saturated fats in the American diet (Eckel et al. 2007). The majority of the possible trans-fat alternatives are already being put to use in some capacity and most will likely experience major growth in the coming years (Eckel et al. 2007). However, due to the nature of the oil production industry, lead time, or the time between planting decisions and oil delivery, becomes a significant hindrance to rapid scale ups for many of these alternatives, particularly for trait enhanced varietals (Eckel et al. 2007). With the average cycle of 4 years lead time, scaling up of trait enhanced varietals of soybean, sunflower, and canola becomes challenging as a wide range of difficult to plan for problems come into play without significant foresight (Eckel et al. 2007). Some of these problems include: securing contracts for field plots housing trait enhanced varietals, preserving the identity of the trait enhanced oilseeds, and additional costs to oils processors related to the need to collect, refine, and store the oil separately (Eckel et al. 2007).

Along those same lines, the diversification of the edible oil profile that will be required by reformulation precluding trans-fatty acids necessitates equivalent diversification of the storage and transportation chains servicing the oil supply chain (Eckel et al. 2007). Without PHOs and without partially hydrogenated soybean oil in particular, the edible oil profile of the U.S. will necessarily diversify greatly and it will behoove food manufacturers to work together to ensure the reliability of their supply chains and the quality of the oils they need for certain types of products, while other partnerships might
be necessary for other types of oils that are suitable for other types of products (Eckel et al. 2007). Likewise, oil manufacturers will need to purchase new storage vessels suitable for holding oils other than partially hydrogenated soybean oil (Eckel et al. 2007). Packaging must be updated to accommodate the stability change over from PHOs. Labels, obviously, must change. In many ways, the reduction of trans-fatty acids is largely a business decision, one that requires coordination and communication above all else (Eckel et al. 2007).

One of the major problems in addressing issues involving trans-fatty acids is the lack of consumer understanding regarding dietary fats. In an online survey conducted by the American Heart Association in 2006, a national sample of 1000 adults aged 18-65 the vast majority of respondents recognized the term “trans-fats” but only about half of them understood the health effects associated with their consumption (Eckel et al. 2007). In order to achieve the consumer driven push for health and wellness focused reformulations, continued awareness and education is needed. One part of the problem that would be easily fixed is consolidation of terminology, because whether they are called trans-fats, trans-fatty acids (TFAs), partially hydrogenated oils (PHOs), industrially-produced trans-fatty acids (IP-TFAs), or something else, the fact remains that trans-fats as a product of hydrogenation pose a serious public health risk. Combating the lack of understanding surrounding this issue has been one of the major hurdles from the start and continues to be going forward.
1.1.1 Hydrogenation and Modified Hydrogenation

One of the major reasons for the delays with regard to trans-fat regulation over the last decade and, more specifically, over the last year has been the oil manufacturing industry and its researchers’ assertions that cleaner, efficient hydrogenation is coming. There have been numerous studies on the hydrogenation process itself over the last 15 years aimed at manipulating the process in such a way that produces a lesser percentage of trans-fatty acids when compared to cis-fatty acids (Veldsink et al. 2008; Dijkstra 2006). In recent years, promisingly low yields for trans-fat as a product of modified hydrogenation procedures have been accomplished by many research groups. Before getting into some of the results of these studies, it is worthwhile to investigate the hydrogenation process itself.

The hydrogenation reaction adds hydrogen to double bonds of polyunsaturated and monounsaturated oils with the intent of altering the melting properties of the resulting lipids (Fennema, 2008). Another way of saying this is that the reaction increases the saturation of the reacted lipids. The reaction requires hydrogen as substrate, temperature control to provide heat initially, ensuring that all lipids are liquid, then to cool the reactants once the exothermic reaction begins, and metal catalyst to speed up the reaction (Fennema, 2008). The most common catalyst is reduced nickel at a concentration of 0.01-0.02%; the nickel is incorporated into a porous support to allow the nickel to be recovered after the reaction finishes using filtration (Fennema, 2008). The reaction takes place between 100°C and 300°C, but higher temperatures favor the formation of trans-
fatty acids by a mechanism that will be discussed hereafter. The reaction takes 40-60 minutes, and the process is monitored by changes in refractive index (Fennema, 2008).

The reaction mechanism involves catalyst initially complexing on either side of a double bond. These complexes are then broken by the adsorption of hydrogen at one side of the double bond, then the other. If there is no hydrogen present to the catalyst associated double bond, the reverse reaction will take place, reforming the double bond. This reformed double bond may be one of two geometric isomers, either the cis or the trans form. The double bond may also migrate to the set of carbons on either side of the double bond’s initial location, the result being a positional isomer of the initial lipid (Fennema, 2008).

The ideal conditions for minimal formation of trans isomers is one at which the reaction temperature is kept low, the reactants are thoroughly agitated, and the pressure applied by hydrogen is matched to the catalyst concentration. The basis of these parameters is to avoid the reformation of double bonds once a double bond-catalyst complex has been formed (Fennema, 2008).

In the past, when reactions were designed to increase the stability of the final product above all else, manufacturers took advantage of catalysts’ greater affinity for pentadiene double bond systems and often increased catalyst concentration while reducing hydrogen concentration in order to favor the sequential saturation of polyunsaturates above
monounsaturates even more (Dijkstra 2006). That this reaction modification also produced excess trans isomers was a desirable side effect (Dijkstra 2006). In the modern era in which the hydrogenation reaction depends on minimizing trans-fat production to survive into the next decade, the types of modifications that are being investigated are nearly the opposite of the modification discussed above (Fennema, 2008; Veldsink, Bouma, & Schöön 2008; Dijkstra 2006).

As previously stated, a great amount of research is currently underway that is seeking to minimize the production of trans-fatty acids as a result of hydrogenation. Reactions with hydrogen pressure around 65psi and temperatures around 70ºC in a traditional slurry reactor using platinum catalyst have been shown to yield as little as 10% TFA by weight with only 14.5% saturates by weight (Singh et al. 2009). Using the same reaction conditions and a high performance integral-asymmetric polyetherimide membrane with platinum interspersed throughout, as little as 4% by weight TFA and 14.5% saturates by weight were achieved (Singh et al. 2009; Singh et al. 2010). The majority of this kind of research will be proprietary until it has been patented, but continued advancement in this field is assured.

Other studies in recent years have focused on the possibility of enzymatically hydrolyzing TFAs from their glycerol backbone. One study found that even using relatively crude enzymatic treatments, hydrolysis of 73.3% of TFAs could be achieved at relatively low cost (Jala et al. 2013). Such findings open the door for combination
treatments involving modified hydrogenation in conjunction with enzymatic hydrolysis in order to achieve 0 trans-fat from partially hydrogenated vegetable oils.

1.2 Instrumentation

1.2.1 Infrared spectroscopy

Spectroscopy is the study of the interaction between light and matter. When matter is acted upon by electromagnetic radiation, it can be absorbed, transmitted, or reflected (Colthup et al. 1990). Each of these possible interactions between a substance and light are used as the basis of one or many spectroscopic methods for quantitative or qualitative analyses. The most common spectroscopic methods are based on the absorption or emission of radiation in the visible (Vis), ultraviolet (UV), radio (nuclear magnetic resonance), and infrared (IR) frequency ranges. Use of the infrared region of the electromagnetic spectrum has seen wide application in food products for the determination of moisture, lipid, protein, and carbohydrate content.

The IR spectrum is subdivided into three regions: the near-infrared (4000-14000 cm\(^{-1}\) wavenumbers), mid-infrared (400-4000 cm\(^{-1}\)), and far-infrared (40-400 cm\(^{-1}\) ) (Colthup et al. 1990). As energy is directly proportional to frequency, light with longer wavelengths, such as the far-infrared region, are of lower energy as compared to the much higher energy near-infrared region. The great contrast in the energy conducted by radiation of these three regions results in great differences in the analytical methods that are available to the researcher and the type of information that can be obtained.
For studies utilizing the infrared region of light, absorption is the primary interaction of interest. When IR radiation (heat) interacts with matter, a portion of the radiation is absorbed by chemical bonds of the sample matter resulting in light that is absent certain frequencies (Colthup et al. 1990). The absorbed portions of this light are associated with shifts in the dipole moment of the molecule as a result of molecular vibrations, stretching and contracting of bonds, and bending or twisting of bonds (Maurer et al. 2012). The charge distributions of the energized sample over time are observed as vibrational energy directly proportional to the strength of the energized bonds. Because of this specificity, conserved atomic arrangements (known as “functional groups”) can be identified in any sample, despite the slightly different vibrational modes associated with the unique connectivity and environment of each molecule (Maurer et al. 2012). When functional groups are excited by incident radiation, they can move from the lowest vibrational state ($v=0$) to the first excited energy state ($v=1$). The radiation frequency that causes this energy transition is equal to the initial vibratory frequency of the bond and is known as the fundamental absorption (Colthup et al. 1990). Absorptions of this type are most commonly associated with the mid-infrared region. Molecules that are bombarded with the higher intensity light of the near-infrared region are capable of further energetic transitions ($v=2$ or 3); these transitions are referred to as overtones (Colthup et al. 1990). The infrared region’s sensitivity to changes in structure and conditions make it well suited for analysis of components in a complex matrix. IR spectroscopy has been widely used in the characterization, authentication, and classification of a great variety of edible
fats and oils (Guillèn & Cabo, 1997; Maurer et al. 2012; Yang et al. 2005). Both near-infrared and mid-infrared spectroscopies have been the focus of a large amount of research into the quantification of low concentrations of fats in general and in trans-fat in particular (Mossba et al. 2005; Adam et al. 1996; Azizian & Kramer, 2005).

1.2.2 Mid-Infrared Spectroscopy

Application of mid-infrared spectroscopy is more sensitive to slight chemical changes in foods than is near-infrared spectroscopy due to type of absorptions associated with each and the characteristics of the resulting spectra (Colthup et al. 1990). Where the broad overtone and combination bands of near-infrared spectroscopy are best suited for determination of the major food components such as moisture, protein content, and fat content, mid-infrared spectroscopy is capable of identifying functional groups within the sample. The sensitivity of mid-infrared spectroscopy is such that chemical changes in food components resulting from storage or processing may be monitored through comparative analysis. Utilizing this region of light is well suited to a wide variety of applications because the positioning of the bands are correlated with the energy of the bond and the intensity of absorption bands of functional groups are proportional to the concentration in the matrix (Guillèn & Cabo, 1997). These characteristics in addition to well-separated bands make MIR spectroscopy ideal for qualitative and quantitative analyses (Li-Chan et al. 2010).
1.2.3 Fourier Transform Infrared Spectroscopy

Over the last three decades, Fourier Transform Infrared Spectroscopy (FT-IR) systems have replaced conventional dispersive systems and reinvigorated the field of infrared spectroscopy. Dispersive systems separate incident infrared radiation such that the sample and detector are interacted with by one wavelength at a time. In contrast, FT-IR systems irradiate the sample with a continuous, wideband infrared signal with all wavelengths arriving at the sample then the detector simultaneously (Guillen & Cabo, 1997).

![Figure 1.1](schematic.png)

**Figure 1.1** Schematic diagram of a Fourier Transform Infrared Spectrometer (Sanchonx)

**Figure 1.1** shows a schematic of a FTIR system. In place of the monochromator of a
A conventional dispersive system is a Michelson interferometer. A Michelson interferometer is composed of a beamsplitter, a fixed mirror, and a moving mirror (Smith 2011). In such a system, the radiation incident on the beamsplitter is split to the moving mirror and fixed mirror, is reflected back on the beamsplitter, and is recombined before interacting with the sample. The partial beams recombine at the beamsplitter, resulting in a constructive/destructive interference pattern as a result of the variations in path length caused by the moving mirror (Ismail et al. 1998). The path length variations caused by the moving mirror and the resulting interference pattern are monitored and calibrated with a high precision laser, typically helium-neon. After recombining, the signal reaches the sample and is selectively absorbed before arriving at the detector. The signal that arrives at the detector is called an interferogram; a plot of intensity versus path length from the moving mirror. This plot is then converted by a mathematical transformation function known as the Fourier transform into a typical IR spectrum of absorbance versus frequency (Smith 2011).

In comparison to dispersive systems, FT-IR spectrometers collect better spectra in a shorter time. Collection speed is enhanced greatly through the replacement of a monochromator with a beamsplitter, allowing for the entire IR spectrum to be collected in a single scan. Additionally, the increase in energy throughput gained through scanning the entire IR spectrum simultaneously produces a much higher signal-to-noise ratio in FT systems; signal-to-noise ratio is also increased by repeated scans (Ismail et al. 1999). Greater consistency between spectra and between instruments is achieved through FT
systems maintaining internal laser wavelength calibration, as opposed to the external calibration required by dispersive systems (Juanèda et al. 2007).

1.2.3 Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy

The greatest strengths of FT-IR systems are rapid throughput, accuracy, and flexibility. IR systems are capable of obtaining spectra from a wide range of samples including solids, liquids, and gases. The traditional method of analysis for IR spectrometers is transmittance of infrared radiation through the sample directly. This method of analysis sets forth a variety of sampling issues for complex matrices that have plagued MIR methods (Griffiths & de Haseth 2006). The advent of Attenuated Total Reflectance (ATR) has overcome many of these issues (Li-Chan et al. 2010). An ATR accessory improves the versatility of IR systems, allowing infrared spectra to be obtained from the surface of a material (Günzler & Gremlich 2002). The accessory is placed between the beamsplitter and the detector in the signal path and it consists of a high refraction-index crystal that directly contacts the sample. The IR beam enters the crystal at a predetermined angle, reaches the interface of the crystal and the sample and is completely reflected back into the crystal. At the point of reflection, an evanescent wave is produced that penetrates the sample and is absorbed (Günzler & Gremlich 2002). Although this wave minimally penetrates the sample (between 1 and 4 µm for MIR), multiple bounce ATR devices allow for many contact points between the sample and the IR beam resulting in absorption by the sample at each point of reflection. The IR beam arriving at
the detector has had various frequencies absorbed by the sample via evanescent waves and the resulting interferogram can be converted to an absorption spectrum via the Fourier Transform.

A wide variety of high-refractive index materials have been used for ATR including: zinc selenide (ZnSe), germanium, and diamond. Each of these has unique properties and must be matched to the sample in question; most commonly in order to assure that the refractive index of the crystal is much higher than that of the sample. If the refractive index of the sample is close to or greater than that of the crystal, internal reflectance will not occur. The only other requirement for ATR sampling is that the sample comes into direct contact with the crystal; in some cases this may require the application of pressure to the sample. In addition to pressure clamps, many ATR accessories now include temperature and environmental control units. This high degree of control during sampling has been used to monitor the degradation of an oil over time via the effects of heat or oxygen content (Javni et al. 2000).

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) is one of the two methods approved by the FDA for measuring \textit{trans}-fatty acid content in foods; the other being gas chromatography (Eckel et al. 2007). Gas chromatography is noted for its high degree of accuracy, particularly at very low levels, but this sensitivity comes at the expense of extensive sample preparation and long run times. ATR-FTIR is noted for its rapid throughput and flexibility (Eckel et al. 2007). Most food manufacturers choose to use ATR-FTIR due to its speed and convenience (Eckel et al. 2007).
The intertwining history of ATR-FTIR and trans-fat dates back to the early 1990s at the least (Van de Voort et al. 1992; Van de Voort et al. 1995) and has continued to flourish into the modern day (Kramer et al. 1999; Mossoba et al. 2001; Mossoba et al. 2009; Mahesar et al. 2010; Rodriguez-Saona & Allendorf 2011; Mossoba et al. 2012). The drawback of ATR-FTIR spectroscopic methods as they relate to trans-fat determination has traditionally been the lack of reliability at trans-fat concentrations less than 1% by weight (Eckel et al. 2007). As ATR-FTIR methods have matured, this has been become less and less of an issue; recently, accurate determination at concentrations as low as 0.34% has been reported (Mossoba, Kramer, Azizian, Kraft, Delmonte, Kia, Bueso, Rader, & Lee 2012). The greatest quotient of the advancement in accuracy for these methods has been a result of the refinement and evolution of ATR methods and technology (Mossoba et al. 2009; Mossoba et al. 2012). Modern sophisticated methods utilize ATR devices with as many as 9 contact points with the sample, hereto referred to as bounces (Mossoba et al. 2012).

ATR devices tend to experience increasing sensitivity with increasing bounces for obvious reasons. The more often the incident light comes into contact with the crystal sample interface, the more sample absorption events are incurred (Colthup et al. 1990). Thusly, the spectral data becomes more refined and more consistent with increasing bounces. For this reason, modern ATR devices have continued to rapidly advance the accuracy and reliability of FTIR methods over the course of the last two decades (Mossoba et al. 2012). An additional trend with FTIR at large and ATR-FTIR in particular is for increased portability. Spectroscopic methods utilizing portable systems
have been shown to produce results with equal or greater accuracy that standard benchtop models with the added benefits of increased portability, ease of setup and use, and oftentimes decreased cost (Birkel & Rodriguez-Saona 2011; Mossoba et al. 2012).

In a direct comparison of NIR and MIR, classification models for discrimination of edible oil varieties built with both spectral ranges and the MIR showed significantly higher classification accuracy (Yang et al. 2005). Previous studies have demonstrated that the internal reflection method of ATR-FTIR produces much greater accuracy than transmission infrared official methods, and that when using the ATR-FTIR methods predictions of trans-fat levels are accurate to as low of as 0.34% of total fat (Adam et al. 2000; Mossoba et al. 2012). Increases in computing power have aided the development of spectroscopic method through the application of the Fourier Transform as well as the multivariate analysis tools capable of extrapolating information from large spectral data sets (Ismail et al. 1999). Most studies rely on several multivariate methods to aid in quantification or classification.

1.2.4 Chemometrics and Multivariate Analysis

Due to the complex matrix effects of most food products, the use of multivariate analysis tools is required to derive meaningful relationships from spectral data sets. Multivariate techniques that classify, quantify, compress, or reduce datasets are a significant feature of IR spectroscopy (Karoui & De Baerdemaeker 2007). There are many multivariate
methods, each with distinct types of datasets and outputs to which they are particularly well suited.

Partial Least Squares Regression (PLSR) is a multivariate method used to correlate spectral information with a set of reference variables. PLSR models attempt to explain the maximum variance in both the reference and spectral data sets in the form of a linear equation (Karoui & De Baerdemaeker 2007). The components that comprise the resulting linear relationship are known as “latent variables”. Frequently PLSR models can explain the variation in a dataset with fewer than 10 latent variables; as only the variables that are most important in explaining a model’s variation are used. A PLSR model is constructed for the determination of a particular chemical component of a matrix and the model must be calibrated for each particular matrix. For example, the same model that predicts the trans-fat content of butter would not be expected to reasonably predict the trans-fat content of a potato chip. That said, very robust models might be capable of such tasks. More robust models include more numerous sample sets in the model and therefore, a greater amount of variance can be used in prediction. PLSR methods involving trans-fatty acids have been used in determination of TFA content (Christy et al. 2004; Sedman et al. 1998; Van de Voort et al. 1995; Mahesar et al. 2010) and in the field of lipid chemistry at large (Maurer et al. 2012; Christy et al. 2004; Javni et al. 2000; Guillèn & Cabo, 2002) often with comparable or better reproducibility than traditional methods.
Chapter 2: Application of Mid-Infrared Spectrometers in Determination and Quantification of Trans-fatty Acid Content in Snack Foods and Bakery Products

Alex M. Milligan, Luis E. Rodriguez-Saona, and Marçal Plans

Department of Food Science and Technology

The Ohio State University

110 Parker Food Science Building

2015 Fyffe Court

Columbus, OH 43210
2.1 Abstract

Nutritional labels often under report trans-fat content. Due to the health problems associated with consumption of trans-fats, efforts must be made to ensure careful monitoring and enforcement of current guidelines. Recent FDA press releases indicate that trans-fat will be removed from the GRAS substances list in the near future. If such regulation were to be enacted, it would effectively act as a ban on all trans-fats in food. The objective of this study was to isolate and quantify trans-fat content in a variety of local food products reporting some level of trans-fat in the product and approximate the prevalence of misrepresentation of trans-fat levels across several types of foods. Isolation of trans-fatty acids from locally obtained food products was achieved using AOAC Official Method 2000.10 and analysis was performed using the Cary 630 portable FTIR. A standard curve was constructed using trielaidin at varying concentrations. Isolated trans-fats from 40 food products were analyzed; three replicates were run for each product. Spectral data examined using partial least squares regression (PLSR) showed very good correlations ($R^2 > 0.998$) for models produced using both spectrometers. Portable ATR-MIR spectrometers allow for increased flexibility in set up and use while retaining the traditional benefits of FTIR spectroscopy such as rapid throughput, high sensitivity, and large amounts of data per second, making it ideal for regulatory applications and well suited to quality control applications.
2.2 Introduction

Trans-fatty acids have been a source of ire for public health advocates for many years now, and the health consequences related to their consumption have been well documented; however, it has been over a decade since the last regulation on trans-fat was enacted in the United States and there are still many food products on the market that contain a wide variety of trans-fat concentrations (Eckel et al. 2007). Recently, there has been a renewed surge of interest in eliminating trans-fat from the modern diet and as such, the need for inexpensive, rapid, and accurate analyses also becomes greatly important moving forward.

The public health risk posed by trans-fatty acid consumption is associated only with industrially produced trans-fatty acids (IPTFA), as naturally occurring trans-fats such as the conjugated linolenic acid found in beef and cow’s milk are not known to have a negative impact on human health. IPTFAs are produced by applying hydrogen gas to an unsaturated fat, such as soybean oil, cottonseed oil, or palm oil, in the presence of a metal catalyst resulting in a loss of unsaturations (Fennema 2008). Unless fully saturated, the product fats will contain some amount of cis and trans geometric isomers. The resulting ratio that these fats end up in can be influenced by the type of metal catalyst, reaction pressure, reaction temperature, availability of hydrogen to the reactants, and the original source of the fat (Veldsink et al. 2008).
IPTFA have been linked to a variety of health problems including cardiovascular diseases, infertility, endometriosis, gallstones, Alzheimer’s disease, diabetes and some cancers (Mensink & Katan 1990; Almendingingen et al. 1996; Teegala et al. 2009); in particular, a vast amount of casual linkages have been found between IPTFA consumption and coronary heart disease, which is the number one killer in the United States (Almendingen et al. 1995; Willet et al. 1993). Due to the health consequences relating to IPTFA consumption, there has been increasingly strict regulatory control over the inclusion of IPTFAs bound for human consumption in many countries over the last 15 years. In November 2013, the FDA announced its plans to remove trans-fats from the GRAS substances list (Food & Drug Administration, 2013). The loss of GRAS status for trans-fat would act as an outright ban as proving that PHOs are unlikely to cause negative health effects would be impossible. This announcement and similar strictures worldwide highlight the need for inexpensive, rapid, and accurate analyses for TFA content to ensure regulatory compliance.

Despite all the negatives surrounding the use of IPTFAs, many food producers chose to use them. Initially, the primary impetus for their use was an economic one; IPTFAs could provide similar baking properties as animal fats at a fraction of the cost (Remig et al. 2010). As producers and consumers became more accustomed to the unique functionality and sensory characteristics of IPTFAs, they began to be embraced culturally and could be found in products all across the nation and in most American homes during the 70s, 80s, and 90s (Remig, Franklin, Margolis, Kosta, Nece, & Streete 2010). As knowledge of the
public health risk they pose began to make its way into the public eye, both producers and consumers have attempted to curb their use of these harmful fats by replacing them with highly saturated fats from both animal and plant sources, or fats processed in other ways, depending on the product in question (Remig et al. 2010). Reformulating products to exclude trans-fats requires the producer to understand the functionality that the IPTFA is providing and then matching that functionality with a specific fat or oil mixture (Eckel et al. 2007). Certain products will be tougher reformulations as IPTFAs bind strongly to food matrices, conferring textural properties that can be difficult to replicate (Valentina et al. 2010). Additionally, IPTFAs are highly resistant to oxidation. All of this amounts to increased cost for the food producer that is attempting to exclude trans-fat from its products (Remig et al. 2010).

Eliminating trans-fat from the modern diet will demand as much or more from regulatory agencies as it does from the producers (Eckel et al. 2007). Historically, determining the fatty acid content of a given food product required the use of chromatographic techniques using expensive equipment, time-intensive analyses, and limited data outputs. These limitations in conjunction with the economic gains to made from use of even very modest amounts of inexpensive IPTFAs has made the enforcement of regulation quite difficult (Remig et al. 2010). Infrared spectroscopy has shown great promise as an alternative to chromatographic techniques in a variety of detection and quantitation studies for trans-fats and other oils (Guillén & Cabo, 1997; Mossoba et al. 1996; Azizian & Kramer 2005; Mahesar et al. 2010; Mossoba et al. 2012).
ATR-MIR benchtop and portable spectrometers combined with multivariate analysis have been shown to be a powerful tool for determination of trans-fat levels as low as 0.34% by weight (Mossoba et al. 2012). Fourier-transform infrared (FT-IR) spectroscopy equipped with ATR accessory yield high signal-to-noise ratio spectral data with limited sample prep, rapid throughput, great flexibility, and less capital cost compared to chromatographic equipment (Mossoba et al. 2010).

The objective of this study was to develop a model to rapidly determine trans-fatty acid content using benchtop and portable FTIR-ATR in combination with multivariate analysis.

2.3 Materials and Methods

Snack foods and baked goods were purchased from local grocery stores and local restaurants in Columbus, OH. These samples were homogenized; the fat was extracted and subsequently analyzed, then included in the multivariate model used for trans-fat content predictions. All fatty acid standards were purchased from Nu-Check Prep Inc. (Elysian, MN). All chemicals and solvents used in this study were purchased from fisher Scientific (Waltham, MA).
2.3.1 Sample preparation

Once obtained, the samples were broken into small pieces then flash frozen using liquid nitrogen. The frozen samples were then blended to produce a finely ground powder. Powdered samples were gravimetrically weighed into cellulose thimbles before undergoing a heated hydrolysis extraction with petroleum ether as a solvent as in AOAC Official Method 945.16 (AOAC, 2009). For each sample, duplicate extractions were performed.

2.3.2 Trans Fat Content

Determination of trans-fatty acid content from extracted fats was achieved using a method combining FTIR-ATR with multivariate analysis. Spectral analysis of sample fats was performed at 65°C to ensure the fats are held in the liquid phase. Spectra of both extractions of all samples were analyzed using temperature controlled ATR-MIR benchtop FTIR and ATR-MIR portable FTIR. Sample spectra were correlated to spectral data obtained from C18:1(9t) trielaidin acid standard (NuChek Prep T-240) in C16:0 tripalmitin standard (NuChek Prep 51A-20) and in C18:1(9c) triolein standard (NuChek Prep 53A-25) over the concentrations of 0.5% trans-fat to 40% trans-fat. Predictive models for both benchtop and portable FTIR models are using 3 factors; each with varying weight on sections of the fingerprint region of the mid infrared spectrum, from about 1500 to 700 cm\(^{-1}\), with particular influence coming from the peak most commonly associated with trans-fat which is found at 966 cm\(^{-1}\).
2.3.3 Fourier Transform Mid-Infrared Spectroscopy

Infrared spectral data was collected on an FTIR portable spectrometer (Cary 630, Agilent, Santa Clara, CA, USA), equipped with temperature controlled, 5-bounce ZnSe crystal ATR. Sample fats were heated to 55ºC in an oven prior to being introduced to the ATR stage in a 65µm aliquot. The crystal was allowed to equilibrate to 65ºC prior to all data collection; afterwards spectral data was measured in duplicate. Spectra were collected over a range of 4000-700 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution. Spectra were collected in terms of absorbance and monitored using MicroLab software (Agilent, Santa Clara, CA, USA).

2.3.3 Multivariate Analysis

Partial Least Squares Regression (PLSR) analysis was used to predict trans-fat content for all samples. Predictions for trans-fat content were achieved by correlating sample spectra to spectra of standard solutions. Normalization and 2\(^{nd}\) derivative transforms were applied to all samples. PLSR models were validated using a cross-validation, leave-one-out approach, and the goodness of fit was evaluated by means of standard error of calibration (SEC), standard error of cross-validation (SECV), coefficient of correlation (\(R_{EV}\)), and relative percent difference (RPD) which is the ratio between the standard deviation of the reference data and the SECV. Multivariate analysis was accomplished using Pirouette 4.0 rev. 2 (Infometrix Inc., Bothell, WA).
Chapter 3: Results and Discussion

Alex M. Milligan, Luis E. Rodriguez-Saona, and Marçal Plans

Department of Food Science and Technology
The Ohio State University
110 Parker Food Science Building
2015 Fyffe Court
Columbus, OH 43210
3.1 Results and Discussion

3.1.1 Determination of Trans Fat Content

ATR-FTIR spectral analysis in combination with PLSR modeling has been previously shown to be a viable tool for predicting trans-fat content in a variety of products (Mossoba et al. 2009). This study aims to validate those results and to show that similar accuracy could be achieved using portable infrared systems. To begin this process, a frame of reference for the relationship between trans-fat content and spectral data had to be established. This was accomplished by producing standard solutions of with trans-fat content ranging from 0.5% to 40% in both tripalmitin and triolein. The two sets of standards solutions were employed in order to ensure that matrix effects of trans-fat being immersed in either highly saturated or highly unsaturated fats were accounted for within the variance of the model. Shown below, Figure 2A and 2B are the relationships drawn from spectral analysis of these two sets of standard solutions.
Figure 2. Partial least squares regression (PLSR) of trielaidin in tripalmitin and triolein over the range of 0.5 to 40% trans using the mid-IR ATR benchtop and portable spectrometers (A-benchtop, B-portable).

As seen in Figures 2A and 2B, both the benchtop and portable systems were able to produce very strong correlations between the reference values measured during standard solution preparation and the predicted values obtained from spectral analysis. The calibration model built from spectral data obtained from the benchtop system shows a slightly higher goodness of fit, but both models produced r-values above 0.99 making them excellent references for further prediction.
As seen above in Table 2, the predictive accuracy of both regression models appears strong. As mentioned earlier, the r-value of the models are both above 0.99, indicating strong correlation between reference values and measured values. The calibration coefficient, $r^2$, is the preferred fitness measurement for this type of study due to the interest in concentration determination; both models shows excellent goodness of fit. The standard errors of cross validation (SEV) for the benchtop and portable models are 0.6 and 0.644, respectively, indicating good robustness in the model. Lower SEV values indicate stronger and more robust models that are unlikely to have problems of over fitting. Models with too few data points for the calibration, or training, set tend to fit their calibration set too tightly and aren’t capable of accommodating for the variance associated with their validation set or that of a real world problem. Error associated with cross validation, SEV, is always higher than calibration, SEC, but models which limit additional error from cross validation are considered much stronger. Having limited variation between these two error metrics indicates that the model will be able to produce

<table>
<thead>
<tr>
<th></th>
<th>Benchtop</th>
<th>Cary</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEV\text{a}</td>
<td>0.600</td>
<td>0.644</td>
</tr>
<tr>
<td>$r$ Value\text{b}</td>
<td>0.9988</td>
<td>0.9982</td>
</tr>
<tr>
<td>SEC\text{d}</td>
<td>0.577</td>
<td>0.624</td>
</tr>
<tr>
<td>$r$ Cal\text{e}</td>
<td>0.999</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Table 2. Statistical analyses for PLSR models developed to determine trans-fat content using both mid-IR ATR benchtop and mid-IR ATR portable spectrometers.

\text{a} Standard Error of Cross Validation  
\text{b} Correlation of Calibration Model  
\text{d} Standard Error of Calibration  
\text{e} Calibration coefficient ($r^2$)
reliable predictions across of variety of matrix effects. This infrared model for *trans*-fat determination would be suitable for quality control and regulatory applications.

The discrimination for the calibration models is based on several specific regions of the fingerprint, 1500 to 700 cm\(^{-1}\), which is a subset of the mid infrared spectra ranging from 4000 to 700 cm\(^{-1}\). The greatest contribution to the predictive strength of the calibration models comes from the peak at 966 cm\(^{-1}\). This peak has previously been shown to be strongly associated with *trans*-fat content and is capable of acting as the sole source of variance within the calibration model with reasonable accuracy (Walker et al. 2007). Other, broader areas of the fingerprint region provide further reliability and predictive accuracy.

**Figure 3.** Spectral comparison of standard solutions on both benchtop and portable mid IR ATR-FTIR spectrometers (A-benchtop trielaidin in triolein, B-benchtop trielaidin in tripalmitin, C-portable trielaidin in triolein, D-portable trielaidin in tripalmitin)
Figure 3: Continued
Shown above in Figure 3 A through D are spectra obtained from the calibration data set on both benchtop and portable spectrometers. Differing levels of trans-fat are shown among the four figures to give a wide sampling of spectra and to ensure clarity. At the top of the figures, the distinctions given to the various regions of the mid infrared spectrum are labelled for frame of reference. The starting point for most spectral analyses is relative bond strength, which is closely correlated with absorption frequency. Stronger bonds have absorptions at greater wavenumbers. As such, the peaks of the fingerprint region, 700-1500 cm\(^{-1}\), are single bonds and tend to be weaker than those of the adjacent double bond region at 1500-1800 cm\(^{-1}\), which in turn are weaker than the triple bonds found at 2100-2300 cm\(^{-1}\). In generating two sets of calibration standards, the goal was to train the system to account for trans-fat being solubilized by both highly saturated and
highly unsaturated fats. This is necessary largely because of the interfering absorptions of saturated fats between 956 and 962\text{cm}^{-1}, which artificially increase the absorption at the primary trans-fat absorption at 966\text{cm}^{-1}. This peak is labelled on all graphs as it contributes the greatest discrimination to the calibration model. Other sources of discrimination within the models lie in less distinct areas of the fingerprint region, generally including the 966\text{cm}^{-1} in addition to adjacent regions.

As previously stated a simple linear regression model built around the 966\text{cm}^{-1} peak that is so strongly correlated with trans-fat content can achieve admirable prediction accuracy. Shown below in Figure 4 are prediction models built using the same standard solutions used for the more complicated multivariate model, but utilizing spectral data from only one peak, 966\text{cm}^{-1}. 
As shown above, the linear fit of trans-fat content of the standard solutions and their absorbance at 966 cm$^{-1}$ is very good for both benchtop and portable systems. These simpler models show good accuracy, but less than the corresponding multivariate models. As can be seen on the figures, the trend lines do no pass through 0. This is due to the background absorbances associated with saturated fats that artificially increases the
absorption at $966 \text{cm}^{-1}$. This background absorbance is a significant source of error for such a simple linear regression model.

<table>
<thead>
<tr>
<th></th>
<th>Benchtop</th>
<th></th>
<th>Cary</th>
<th></th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>Simple</td>
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<td>rCal$^d$</td>
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<td>0.994</td>
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</tbody>
</table>

**Table 3.** Comparison of statistical analyses for linear regression models and PLSR models developed to determine trans-fat content using both mid-IR ATR benchtop and mid-IR ATR portable spectrometers

$^a$ Correlation of Calibration Model  
$^b$ Standard Error of Calibration  
$^d$ Calibration coefficient ($r^2$)

Shown above in **Table 3** is an expansion of Table 2 comparing the predictive accuracy of simple linear regression models to their multivariate counterparts. The models exhibit similar correlation metrics, but the simple models include more than twice the error. This more simplistic approach involving integration of the 2nd derivative of the $966 \text{cm}^{-1}$ absorption band is the currently preferred ATR-FTIR standard method by the FDA for *trans*-fat determination, and as such, determination below 1% of total fat is considered beyond the scope of spectrophotometric analyses. Assuming multivariate methods gain wide acceptance and application, these simple linear regression models could still be a useful tool for initial screening in quality control or regulatory applications, but it must be acknowledged that they are susceptible to error as a result of the effects of complex matrices, varying forms of *trans*-fat, and the background absorbance of saturated fats.
being counted as \textit{trans}-fats. A model built around such a narrow range of spectral data lacks robustness and therefore flexibility to accurately predict low levels of \textit{trans}-fat in complex matrices. However, the relative accuracy of these simple linear regression models highlights the statistical strength of building a more robust multivariate model with the 966\ $cm^{-1}$ peak as its basis. Including other spectral ranges within the fingerprint region of the MIR spectrum allows the models to accurately predict \textit{trans}-fat content within a wide variety of complex food matrices.

Among the food products analyzed during the course of this study, the vast majority of foods containing \textit{trans}-fat were predicted to contain \textit{trans}-fat levels very close to their labelled amount. All of these products, their labelled \textit{trans}-fat content and the \textit{trans}-fat content predicted by both the benchtop and portable MIR spectrometers are shown below in Table 4.
<table>
<thead>
<tr>
<th>Food Products</th>
<th>Estimated benchtop \textit{trans} per serving (g)</th>
<th>Estimated portable \textit{trans} per serving (g)</th>
<th>Labelled Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cookies</strong></td>
<td></td>
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</tr>
<tr>
<td>Ginger Lemon Crème</td>
<td>1.7</td>
<td>1.6</td>
<td>2</td>
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<td>Cheesecake Middles</td>
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<td>0.3</td>
<td>0</td>
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<td>0</td>
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<td>5</td>
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<td>0.5</td>
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<td>0.6</td>
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<tr>
<td>Chocolate Marshmellow Pie</td>
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<tr>
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<tr>
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<tr>
<td>Glazed</td>
<td>2.6</td>
<td>2.3</td>
<td>2</td>
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*Table 4. Predictions for Trans-fat Content of Various Snack Foods and Bakery Products Using Both Benchtop and Portable ATR-MIR Spectrometers and Partial Least Squares Regression.*
As seen above, predictions between models agree with one another very well across almost all samples analyzed during the course of this study. Variations seen between the predictions produced by the differing models are generally very low with only 7 of the 38 samples showing greater than 0.2 g discrepancy between the two models. Among samples that report 0 g trans-fat there appears to be two current trends in the market. The first is to use 0.1 to 0.3 g of trans-fat per serving, likely for functional characteristics trans-fat is able to impart at very low concentrations. A second trend found during this study is for products to use 0.4 to 0.6 g per serving in order to maximize the impact of trans-fat functionality and mouthfeel without having to label any trans-fat. Obviously, for these products that find themselves at the border, the allowance for 20% batch variance becomes very important. Among the samples analyzed during this study labelling some amount of trans-fat some products were found to have trans-fat content at levels very close to their labelled amount, others significantly above, and still others significantly below. Samples that are underreporting were found to be primarily sold in places that do not display nutritional information such as bakeries and local shops. Samples that are underreporting tend to be products that are manufactured by smaller companies that uses a large amount of trans-fats and, thusly, are susceptible to batch variations from their oil supplier and must compensate by overestimating their trans-fat content.
3.2 Conclusions

Temperature controlled ZnSe ATR-MIR benchtop and ZnSe ATR-MIR portable spectrometers have proven to be fast, reliable, and rapid tools for use in the detection and quantitation of trans-fat content in a variety of snack foods and bakery products. With strong correlation coefficients ($R^2 > 0.998$) and minimal error ($SEV < 0.65$) partial least squares regression models were able to accurately predict trans-fat content in a variety of complex matrices. The portable spectrometer produced comparable results with slightly greater error than the benchtop spectrometer. The utilization of ATR-MIR spectroscopy in combination with multivariate analysis was found to be a powerful tool suitable for the detection and quantitation of trans-fat content in applications of both regulatory and quality control; portable spectroscopy was similarly found to be well suited to such applications.
3.3 References


Food and Drug Administration. 2013. FDA takes step to further reduce trans fats in processed foods. FDA News Release.


Mossoba, M., J. Kramer, V. Milosevic, M. Milosevic, and H. Azizian. 2007. Interference of saturated fats in the determination of low levels of trans fats (below 0.5%) by infrared spectroscopy. *Journal of the American Oil Chemist's Society* 84: 339-42.


