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Heat Induced Cis/Trans Isomerization in Vegetable Oils and Oleic Acid

John Harwood Scott
Longwood University

Sarah E.G. Porter
Longwood University

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Spring Honors Thesis

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John-Harwood Scott and Sarah E. G. Porter

Department of Chemistry and Physics, Longwood University, 201 High Street, Farmville, Virginia 23909.

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Abstract

With the FDA mandating that all foodstuff labels list the amount of *trans* fats within their product, it becomes necessary to have a rapid and reproducible method for quantifying *trans* content within foods. Research has led to the advent of an analytical method that utilizes attenuated total reflectance with a Fourier transform infrared spectrometer to quantitatively measure *trans* fat in foodstuffs. The method is possible because of an infrared absorbance band at 966 cm^{-1} that is unique to nutritional *trans* fats. This research used the aforementioned method to quantify *trans* fat content in common cooking vegetable oils. Before analysis, the oil samples were heated to different temperatures that were less than or equal their respective smoke point. Results from all heated oil samples provided data that was below the calibration range (0.5 – 40% *trans* fat).

Introduction

In order to improve taste, shelf-life, and cooking quality at a low cost, the food industry has long been in the practice of converting vegetable oils into margarines and shortenings, via a hydrogenation process, that are to be used primarily in most packaged foodstuffs.[1] Fatty acids, the main constituents of vegetable oils, are a carboxylic group attached to a long hydrocarbon tail of varying length.[1] Saturated fatty acids have no double bonds in the tail while mono- and polyunsaturated fatty acids have one or many respectively.[1] A saturated and a polyunsaturated fat are shown in Figure 1a-b. The linear geometry of saturated fats allows them to compact and be in a solid state at room temperature. The bent geometry of unsaturated fats inhibits compaction and makes them liquid state at room temperature.[1]

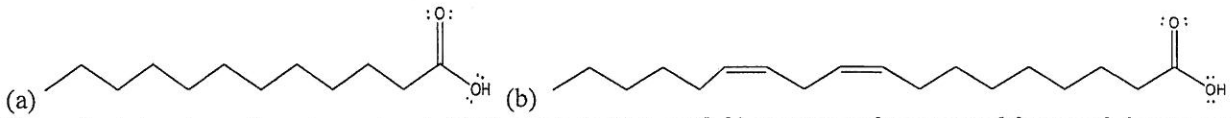

Figure 1 *a) structure of a polyunsaturated fatty acid: linoleic acid; b) structure of a saturated fatty acid: lauric acid*

During hydrogenation, the double bonds present in the polyunsaturated and monounsaturated fatty acids are converted to saturated fatty acids.[1,2] It is a simple process that consists of mixing heated vegetable oil with hydrogen gas in the presence of a catalyst.[1] Flavor stability and longevity is achieved with hydrogenation because, in reducing the amount of double bonds, the process limits possible sites for oxidation.[1] Complete hydrogenation requires ample energy input through catalyst concentration and pressure and does not always provide the desired functional characteristics in the oil product.[3]

In order to elicit the desired characteristics from hydrogenated oil, the food industry implements a selective hydrogenation process.[1,2,4] The process is selective in that the catalyst used hydrogenates fats with the highest degree of unsaturation first (poly-) before hydrogenating

di- or monounsaturated fats.[1] This allows more control over the level of hydrogenation within the oil product.[1] The vegetable oils are modulated by temperature, pressure, agitation, and catalyst concentration during hydrogenation to keep desired quantities of unsaturated fats in the final product. The remaining unsaturated fats give the hydrogenated oil a better liquid to solid fat ratio and therein, better funtionality.[1]

During hydrogenation, the natural *cis*-geometry carbon-carbon double bonds of the unsaturated fatty acids in vegetable oils are broken.[5] The freed electron density goes toward adding hydrogen to both the carbons that had previously been double bonded.[3] This process, like any other reaction, is expedited with heat and is rapidly facilitated by a two-step nickel catalyst.[2,3] However, in partial hydrogenation, where there are unsaturated fats that remain throughout hydrogenation, the double bond can break from the heat or an incomplete interaction with the catalyst and subsequently reform.[3] When reformation occurs, the *trans* geometry of the double bond is strongly favored over the natural *cis* for both thermodynamic and steric reasons.[3] The resulting fatty acid by-product is then termed a *trans* fat.[1,2,3] Since *trans* fats are linear in geometry, like a saturated fat, they increase the solid ratio of the hydrogenated oil. A comparison of a *cis* and *trans* fat is shown in Figure 2 a-b. Margarine and shortening stocks produced commercially from vegetable oils can have *trans* fat concentration as high as 40%.[2]

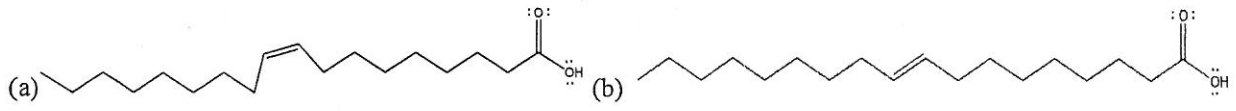

Figure 2 *a) structure of cis oleic acid; b) structure of trans oleic acid or elaidic acid*

The formation of *trans* fatty acids during the production of partially hydrogenated oils in the food industry is a negative consequence due to the adverse health effects that have been attributed to dietary *trans* fats.[2,5,6,7] In particular, initial research showed that increases of dietary

trans fats gave rise to significantly higher risks of coronary heart disease (CHD) in humans.[2,5,6,7] More clinical research and trials followed to investigate why *trans* fats contribute to CHD, and subsequently atherosclerosis.[2]

Human digestive enzymes that specialize in the uptake, transport, and degradation of fatty acids are shape-specific to the natural, *cis* double bond formation.[5] Without uptake or degradation, an ingested *trans* fat will not have the metabolic or structural potential of the original fatty acid.[5] Furthermore, *trans* fats mimic the shape of a saturated fat.[5] Body cells can attempt to incorporate *trans* fats into structures, like membrane phospholipids, resulting in a deficiency of said structure to perform its desired task.[5]

With respect to CHD, *trans* fats show their most adverse effect on the levels of blood lipid carrier molecules: low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol.[5,6,7,8] Both molecules are measured by the ratio of their respective concentration in the blood.[5] A high percentage of LDL (the *unhealthy* lipid cholesterol) in comparison to HDL (the *healthy* lipid cholesterol) has long been the standard indicator for both CHD and an elevated risk for atherosclerosis.[5] Saturated fats, which are also considered unhealthy, raise LDL levels but leave HDL levels constant.[5] *Trans* fats however, not only increase the amount of LDL but decrease the amount of HDL in the blood, leaving a cholesterol ratio that is far worse than what saturated fats produce.[5,8] Such malevolent health effects found in epidemiological and cellular studies brought *trans* fat to the attention of the public, food suppliers, and government regulation agencies.[4]

The United States' Food and Drug Administration (FDA) has the responsibility to guarantee that products intended for human ingestion are safe for consumption.[4] The jurisdiction of the FDA is outlined primarily in the Fair Packaging and Labeling Act and Federal Food, Drug,

and Cosmetic Act (FFDCA); both of which provide the federal laws necessary to inspect and regulate the safety of almost all U.S. foodstuffs.[4] In 1990, the Nutrition Labeling and Education Act sought to expand the required nutritional elements, previously listed in the FFDCA, that are required to be present on the labels of all foods and supplements. However, when the FDA implemented its final rule in 1993 and turned the act into law, *trans* fats were not included in the amended list of required nutrients for labeling purposes.[4] At the time, the FDA considered research on the health implications of *trans* fats to be incomplete, making it premature to require the amount of *trans* present to be listed on a label.[4]

In light of more and more research that corroborated the findings outlined above concerning the negative health effects of *trans* fats, the FDA was forced to revisit the issue of trans fat labeling.[4] Expert panels were convinced by the scientific evidence and were strongly suggesting that the American consumption of *trans* fats should be limited.[4] Their recommendation, in parallel with old recommendations concerning saturated fats, was that consumers should purchase foods that had low, if any, *trans* fat content.[4] The FDA conceded to both the experts and a growing public outcry in 1999. In 2003, a final rule passed which mandated that the *trans* fat content of all foods and supplements be listed, starting in 2006.[9] As dictated in the final rule, companies are obligated to list the exact *trans* fat content, unless it is less than 0.5 g / serving size in which case *trans* content can be labeled as zero .[9]

With the labeling of *trans* fat content mandatory, it became necessary for regulators and manufacturers to have a simple, rapid, and reproducible method for assessing *trans* content in foodstuffs.[2,10-13] As food manufacturers attempt to reduce and limit the amount of *trans* fat in their product, the analytical method employed must also be particularly sensitive to quantify lower concentrations (e.x % *trans* $\leq$ 5% *trans* fat) in order to ensure label accuracy.[4,10-14]

A capillary gas chromatography (GC) method was initially used to quantify *trans* fat in food samples.[4,10-14] An official GC method was approved and released by the Association of Official Analytical Chemists (AOAC).[4] Accurate and reproducible determination of *trans* fat content and identification of each specific *trans* fatty acid present are both very possible with the GC method.[4,10-13] However, GC has limitations in its use for regulatory purposes.

The use of a long (100 m) capillary column coated with a highly polar stationary phase is required to achieve the needed separation of every fatty acid.[4] Sufficient separations can take as long as 1.5 hours for a single analysis.[4,10,11] Therefore, despite its reliable accuracy and detailed information on fat content, the time involved with sample preparation and analysis makes the GC method inefficient for the quick and repetitive determination of *trans* fats needed for labeling and regulatory oversight.[4,10,11]

Another technique has been tested and implemented for rapid determination of trans fats: infrared spectroscopy (IR).[4,10-14] The IR method relies on the spectroscopic properties of trans fatty acids, particularly a CH out-of-plane deformation band seen at 966 cm^{-1} that represents an isolated trans double bond absorption.[4] This is particularly useful because the band is unique to the specific *trans* double bond that the FDA uses to define a nutritional trans fat: an unsaturated fatty acid that has one or more non-conjugated, *trans* double bonds.[4,9] Fatty acids that posses conjugated *trans* bonds have been shown in research to not have the adverse health effects of isolated trans bonds.[15] In fact, some actually exert positive health benefits.[15,16]

Conjugated double bonds that are found naturally in unsaturated fatty acids and other molecules have absorption bands that are shifted to 985 – 990 cm^{-1} and to 940 – 950 cm^{-1}.[12] Therefore, if a food sample contained heavily conjugated molecules, such as lycopene shown in Figure 3a, or a conjugated fatty acid, like conjugated linoleic acid shown in Figure 3b, absorption

from the conjugated trans bonds would not interfere with the trans absorbance at 966 cm^{-1}. This spectrometric characteristic makes the 966 cm^{-1} band exclusive to nutritional trans fatty acids.[4,10-14]

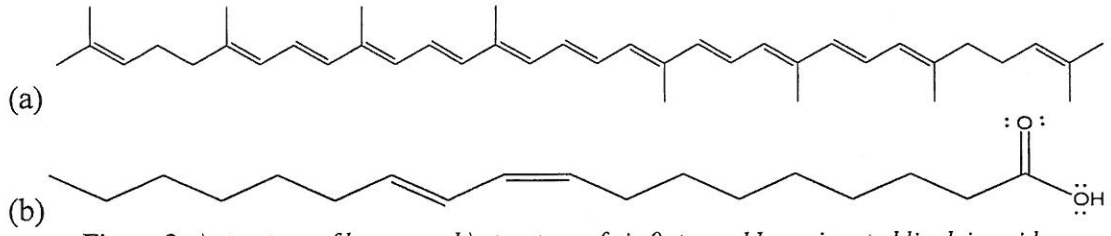

Figure 3 a*) structure of lycopene; b) structure of cis-9, trans-11, conjugated linoleic acid*

Quantification is achieved by standards and a calibration curve constructed from the area under the 966 cm^{-1} band. Integration is used, as opposed to the individual absorbance value at 966 cm^{-1}, in order to fully encompass the infrared absorption intensity.[4,12,13] Unfortunately, the absorption at 966 cm^{-1} occurs over an elevated and sloping baseline, as seen in Figure 4, which gives rise to inaccuracies in quantification via integration, particularly as trans concentration gets smaller.[4,10-14]

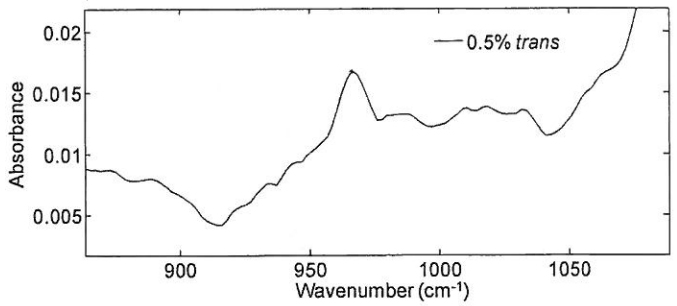

Figure 4 Absorbance spectrum of 0.5% trans fat standard; peak at 966 cm^{-1} is starred

In order to achieve better accuracy and resolution, internal reflection methods are used for infrared absorption analysis.[4,10-14] In particular, attenuated total reflection (ATR) has been commonly implemented because of its ability to analyze a sample that has not been specially prepared or a total lipid extract sample.[4] During ATR analysis, infrared radiation barely

penetrates (only a few μm) the fat or oil sample that rests above an internal reflection crystal.[4,10] As IR light oscillates, undergoing internal reflections, within the crystal, small amounts of radiation penetrates the sample.[4,10] The radiation intensity quickly decays, thus changing the wave's frequency which can be used to measure the depth the wave penetrated.[4,10] Using radiation angles, penetration depth, and Fourier transform, the attenuation of IR within the sample can be calculated and used to form a spectrum that has greater accuracy and better resolution than conventional IR spectroscopy.[4,10] An ATR diagram is shown in Figure 5.

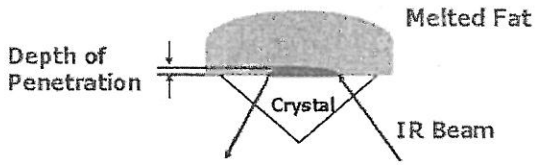

Figure 5 Simple ATR diagram[4]

Spectra from a *trans* free reference oil, ideally of similar fatty acid structure, are used as a background to compare with a *trans* present sample.[4,10] The official AOAC method for analyzing *trans* fats using IR methods recommends ATR with Fourier transform infrared spectroscopy (ATR-FTIR).[4,10-14,14]

The addition of ATR and Fourier transform in the official method did eliminate the baseline offset and slope, yet its accuracy at lower concentrations (less than 5%) was only slightly improved.[4,10] The remaining inaccuracy was attributed to the inability to find a reference fat that is absolutely *trans* free and has a composition that closely matches every test sample.[4,11,12,13] Also, saturated fatty acids were concluded to be causing absorbance interference in the spectra of samples with low *trans* content.[4,12,13]

Nevertheless, further research on the ATR-FTIR method has led to improvements that have increased its accuracy and sensitivity.[4, 12,13] In specific, a negative second derivative method

(-2D) has been implemented successfully and is now nearing the end of a validation process by the AOAC.[4,12,13] The second derivative has commonly been used to enhance spectral resolution, particularly with Fourier spectra.[4,12,13] The negative in the method's name simply means the spectra are multiplied by -1 to have the second derivative absorption peak face up for convenience. Even when *trans* fat standards were analyzed relative to air, as opposed to a reference oil, the -2D method eliminated the sloping baseline, and clearly resolved bands from interfering fats away from the 966 cm^{-1} band.[4,12,13]An absorbance spectrum compared to a second derivative absorbance spectrum is shown in Figure 6a-b. As shown in Figure 6b, the starting and finishing points of the 966 cm^{-1} band is clearly defined. The precision of the -2D method is considered to be 0.5% *trans* fat as a percentage of total fat.[2,15]

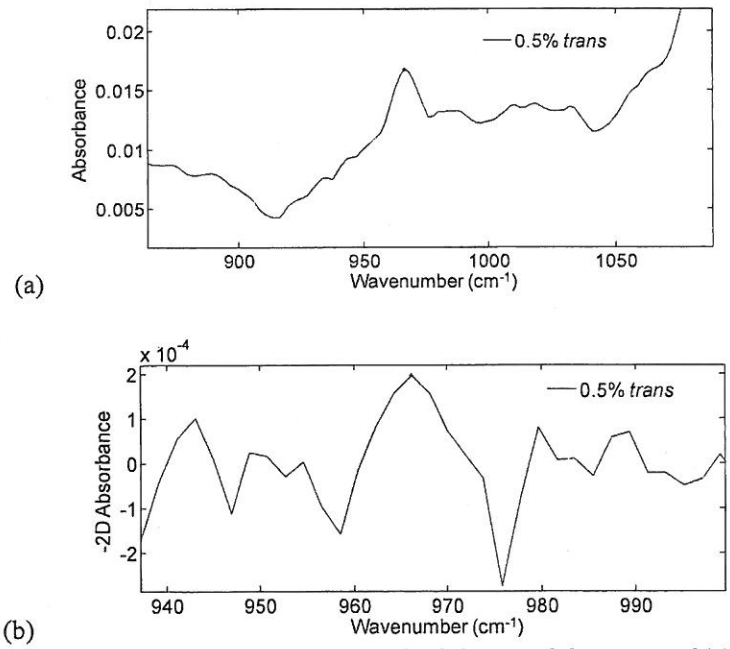

Figure 6 *a) Absorbance spectrum 0.5% trans standard; b second derivative of (a); peak at 966 cm^{-1} is starred*

As indicated by the FDA mandating that *trans* fat content be listed on the label of all foods and supplements, there is considerable public concern relating to *trans*-fats.[17] Consumers

are increasingly more likely to buy products that contain natural polyunsaturated fats, which have been correlated to a lesser risk of coronary heart disease.[17,18] An example of this shift is seen in the purchasing of oiled cooking products.[17] People are now more inclined to cook with a vegetable oil medium, to avoid the saturated fats of butter or margarine and to have the health benefits from the oil's polyunsaturated fats.[17] However, the health benefits of the vegetable oil would perhaps be reduced if the heat and duration of cooking induces *cis*-unsaturated fats to isomerize to *trans,* much like it does in hydrogenation and vegetable oil deodorization processes.[1,19]

This research investigated the effects of heating common cooking oils on their *trans* fat content. Furthermore, oleic acid, which is the greatest monounsaturated fat constituent in canola oil, was also studied in this manner. The hypothesis was that the isomerization of *cis* oleic acid to *trans* oleic acid could be easily analyzed and could conceptually represent what was occurring in the cooking oils. Each oil was heated to a series of designated temperatures below, equal to, or slightly above its respective smoke point. The smoke point is the temperature at which the fatty oil starts produce smoke from internal hydrolysis and oxidation.[1] This temperature is a common indicator of when a fatty oil begins to degrade and lose culinary functionality (like taste).[1] At each temperature, aliquots of each oil were removed at designated times for ATR-FTIR testing. The -2D method was employed to achieve the greatest accuracy in quantifying total *trans* fat concentration in the oils. For the purposes of comparing methods, a fatty acid rich cooking medium was analyzed qualitatively via GC-MS. The results from which greatly emphasized the advantages of ATR-FTIR.

Experimental

ATR-FTIR Method

In accordance with past research that quantified *trans* fat content, standards were prepared using trielaidin (2,3-bis[[(E)-octadec-9-enoyl]oxy]propyl (E)-octadec-9-enoate) as a pure *trans* fatty acid and triolein (2,3-Bis[[(*Z*)-octadec-9-enoyl]oxy]propyl (*Z*)-octadec-9-enoate) as a pure *cis* fatty acid standard.[11,12] Specifically, Nu-Chek Prep trielaidin and MP Biomedicals triolein were used. These fatty acids are identical, except for the geometry of their carbon-carbon double bond. Structures for trielaidin and triolein are shown in Figures 7a and 7b respectively. Standard concentrations ranged from 0.5 – 40% *trans*/*cis* fat w/w.

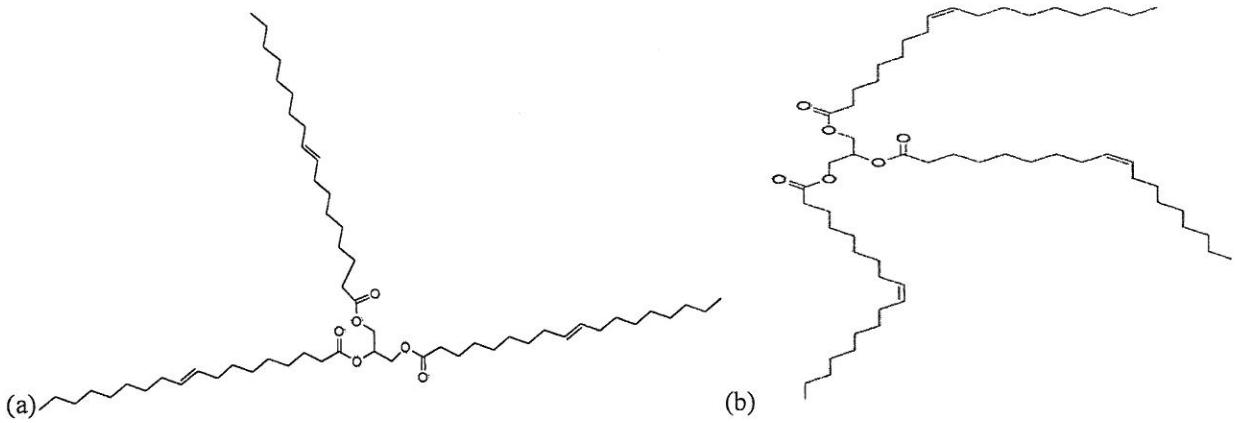

Figure 7 *a)structure of* trans *standard trielaidin; b)structure of* cis *standard triolein*

The specific oils used in this experiment were Fisher® reagent grade pure oleic acid, Great Value® (GV) pure corn oil, GV pure canola oil, GV pure olive oil, and Filippo Berio® olive oil. Except for oleic acid, all oils were purchased at Wal-Mart® which sells Great Value® products as the generic brand. Therefore, the GV pure olive oil was considered the generic brand and Filippo Berio® olive oil the name, or premium brand.

For each heated sample, moderately sized aliquots of the cooking oils were put in a beaker and heated with a Corning PC-420D hot plate. A stir bar was added to each sample to ensure temperature uniformity throughout the oil. Fisher® thermometers, held on a clamp, were used to monitor temperature.

The smoke point of all oils tested is listed in Table 1. For all oils, except canola oil, samples were brought to the smoke point, 50 °C below the smoke point, and 100 °C below the smoke point. Canola oil samples were heated to temperatures starting at 100 °C and going to 240 °C (the smoke point) by 20 °C increments. An additional measurement for canola oil and generic olive oil was taken where an oil sample was heated to 10 °C above the smoke point. The exact temperatures used for each oil is outlined in Table 1.

Table 1 Outline *of oils used, their smoke point, and temperatures samples were heated to*[20]

Oils	Smoke Point (°C)	Sample Temperatures (°C)
Canola Oil	240	100, 120, 140, 160, 180, 200, 220, 240, 250
Generic Olive Oil	216	120, 170, 220, 230
Premium Olive Oil	216	120, 170, 220
Corn Oil	240	140, 190, 240
Oleic Acid	200	100, 150, 200

In order to assess production of *trans* fats at a given temperature over the course of time, small aliquots were removed at different time intervals from each heated sample. Initially, said intervals were 1, 3, 5, 10, and 15 minutes. However, the intervals were modified to 1, 5, and 12 minutes, to save time and materials.

All standards and samples were analyzed using a Thermo Nicolet Avatar 360 FT-IR with Smart MIRacle. The ATR apparatus had a zinc selenide crystal. In accordance to past research, 256 scans were taken per analysis with a resolution of 4 cm^{-1}.[4,10-14]

Absorbance spectra were taken from the FTIR and uploaded into the data analysis program, MatLab® 7.9.0 (R2009b). Matlab did not have pre-written functions to take a second derivative or area under a spectrum curve. A function was written to take both the negative second derivative of all spectra and the area under the 966 cm^{-1}. Peak area was estimated using the trapezoid rule, shown in Figure 8 where 'S(X)' represents the spectral output at any given wavenumber (X), and X_0 and X_n represent the starting and final wavenumbers for integration.

$$Area = \left(\frac{\mathrm{Xn} - \mathrm{Xo}}{2}\right)[S(\mathrm{Xo}) + 2 * S(Xo + 1) + 2 * S(Xo + 2) + 2 * S(Xo + n) \ldots + S(Xn)]$$

Figure 8 *Trapezoid rule for a spectrum (S) from points X_0 to X_n*

The area for the 966 cm^{-1} band was taken from wavenumbers 955 – 974 cm^{-1}. This range is smaller than what was used in past research.[4,12,13] However, the range used encompassed the entire *trans* band of even 40% *trans* standards and still provided a sufficient number of data points for integration via trapezoid rule.

Gas Chromatography-Mass Spectrometry (GC-MS) Method

The cooking medium to be assessed qualitatively was Crisco® All-Vegetable Shortening because it closely matched the shortening used in past research with GC-MS.[21] In order to be compatible for chromatography, the shortening had to be prepped with a lipid extraction and a transesterfication to convert the fatty acids to methyl esters for volatility.[4,21] In accordance with previous research, a 100 mg sample of shortening was dissolved in 25 ml of hexane.[21] Roughly 5 ml of the mixture solution was added to 250 µl of 0.5 M sodium methoxide/methanol solution and vortexed. A 5 ml aliquot of saturated NaCl solution was added. The container was shaken

vigorously and allowed to settle before transferring 3 ml of the hexane layer to another vial to be mixed with sodium sulfate to remove any residual water before GC analysis.

A Varian 3900 GC oven and Varian 2000 GC-MS were used for analysis. A FactorFour™ VF-1ms capillary column (30 m x 0.25 mm, 0.25 μm film thickness) was used out of convenience because it was already connected within the oven. Again, in accordance with past research using GC-MS, the injection volume was 1 μl, injection temperature was 250 °C, and the injection split ratio was 100.[21] The best fatty acid methyl ester separation seen in previous research utilized the following temperature programming: initial temperature 150 °C, hold 10 minutes, ramp at 2.7 °C/min, final temperature 210 °C, hold for 3 minutes.[21]

Results and Discussion

ATR-FTIR Results

Calibration Curve

Spectra from the *trans* standards were successfully analyzed in Matlab using the -2D method. As shown in Figure 9, the *trans* peak of -2D spectra maintained a consistent shape with all standards and increased in height as the percent *trans* increased. However, as seen in Figure 9, some of the -2D absorbance data points in the integration range (955 – 974 cm^{-1}) were negative. In order to avoid using negative data points for integration, the spectra over the integration range was offset so that the lowest point became zero. For consistency, all subsequent spectra from oil samples were offset in the same way.

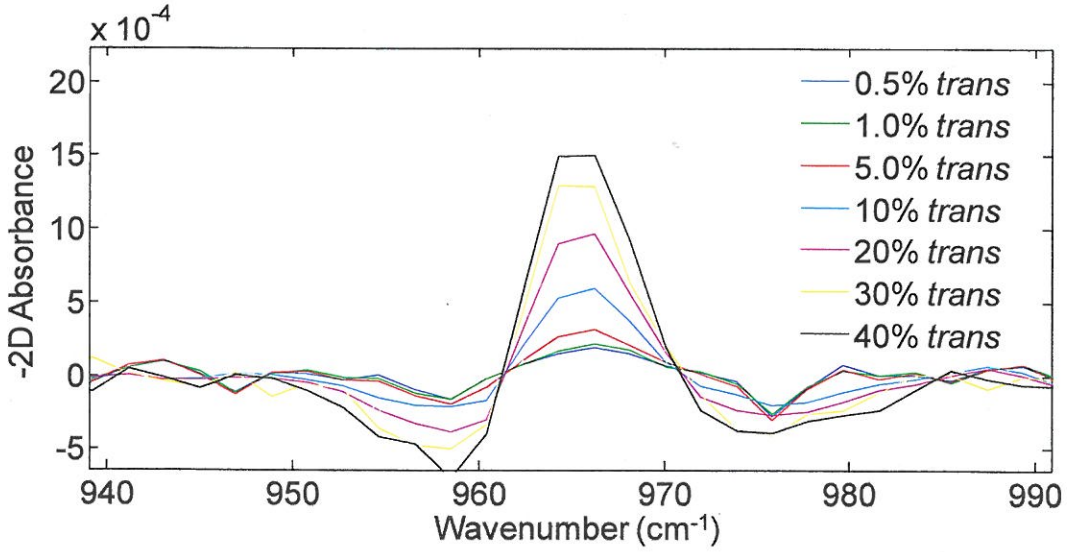

Figure 9 *-2D spectra of calibration standards*

The area of the 966 cm^{-1} band for each standard is listed in Table 2. A calibration curve was composed from this data, as shown in Figure 10. In accordance with the literature, a positive linear correlation was seen between area and % *trans* fat that had a correlation coefficient of 0.9499.[4,10-13]

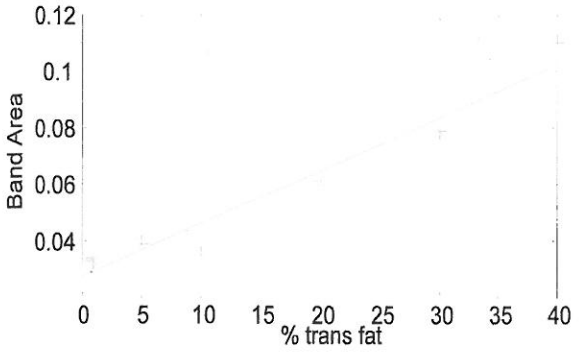

Figure 10 *Calibration curve*

%Trans	Area
0.5	0.034
1	0.033
5	0.039
10	0.035
20	0.061
30	0.079
40	0.11

Table2 *Areas of trans standards*

Vegetable Oil Samples

Canola Oil

The complete area values for all canola samples are listed in Table 3. Regardless of temperature or duration, all areas of the 966 cm^{-1} band were below the calibration range, meaning their band area was below 0.034. Therefore, the *trans* concentration is below 0.5%. As seen in Table 3, there was no correlation between area of the *trans* band with duration of heating for a given temperature. For convenience, all area values for a given temperature were averaged together and are listed in Table 4 in comparison to the *trans* band area of "neat," or unheated, canola oil.

Table 3 *Area under -2D 966 cm^{-1} band for all canola oil samples*

	100°C	*120°C*	*140°C*	*160°C*	*180°C*	*200°C*	*240°C*	*250°C*
1 min	0.008	0.0073	0.0103	0.0107	0.0107	0.0095	0.0079	0.0121
3 min	0.0076	0.0074	0.0108	0.0115	0.0107	0.0096	0.0093	0.0107
5 min	0.0072	0.0062	0.0107		0.0108	0.01	0.0095	0.0119
10 min	0.0084	0.0092	0.0096	0.0109	0.0102	0.0106	0.0096	0.0131
15 min	0.0059	0.0093	0.011	0.0088	0.0109	0.0105	0.0092	0.0098
Average	0.00742	0.00788	0.01048	0.01048	0.01066	0.01004	0.0091	0.01152

Table 4 *Area under -2D 966 cm^{-1} band for all canola oil samples*

Sample	Area
neat	0.0080
100°C	0.0074
120°C	0.0079
140°C	0.0105
160°C	0.0105
180°C	0.0107
200°C	0.0100
240°C	0.0091
250°C	0.0108

Interesting to note, the neat canola oil had a larger *trans* band area than samples heated at 100 °C and 120 °C. This is misleading, considering how the shape of the *trans* band, particularly its width, changed with temperature, as seen in Figure 11. The change in *trans* band shape can be attributed to interference from other fatty acids, particularly saturated fatty acids.[13] This is in accordance with past research which noted that noted that *trans* levels below 0.5% were subject to shifting because the *trans* absorbance at those concentrations is too feeble to outcompete interference from saturated fats.[4,13] Yet, as seen in Figure 12 and 13, the *trans* band started to have a more consistent shape and got more prominent with increasing temperature. Differences in area, however, were minimal. As seen in Figure 14, there was no correlation between temperature and the 966 cm^{-1} band area within the experiments time range.

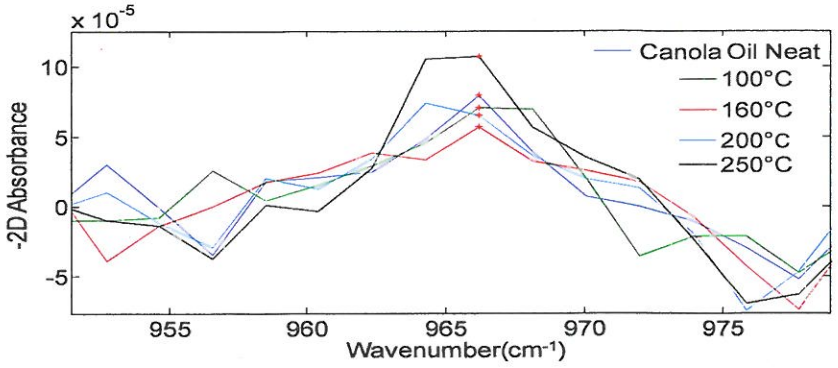

Figure 11-*2D spectrum of choice heated canola oil samples that shows trans band shifting and shape change*

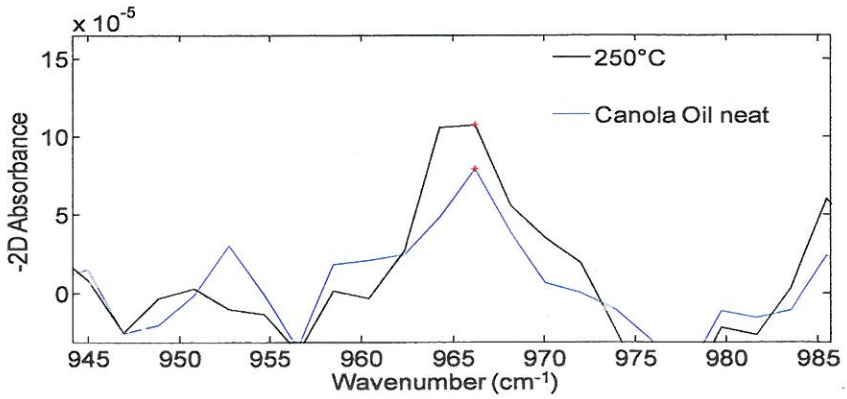

Figure 12 -*2D spectrum of canola oil neat versus the most extreme heated sample (250 °C)*

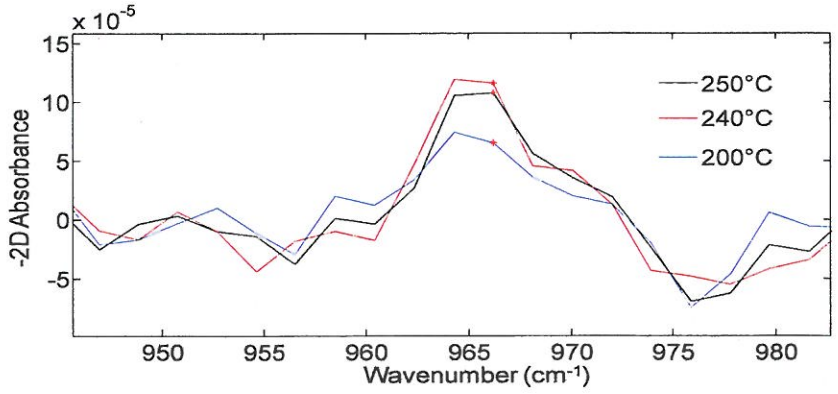

Figure 13-*2D spectrum of the hotter heated samples showing slight band shape uniformity*

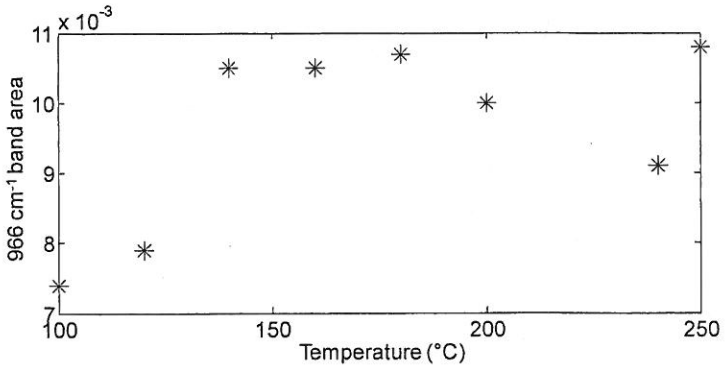

Figure 14 *Graph of average trans band area over time that shows no correlation between them*

Corn Oil, Generic and Premium Olive Oil

The areas of the 966 cm^{-1} band for all corn oil, generic olive oil, and premium olive oil were under the calibration range. Thus the *trans* concentration was below 0.5%. As with canola oil, band areas fluctuated slightly within a given temperature and no correlation was seen between area and increasing time. The areas for all samples within a given temperature were averaged together and are listed in Tables 5-7 in comparison to the *trans* band area of the neat oil. Once again, the areas of the neat oils were higher than some of the heated sample averages. This is still explained by the change of shape the spectra experienced with heating.

Table 4-6 *Areas for the respective temperatures of corn oil, generic olive oil, and premium olive oil*

Table 5	
Sample	**Area**
neat	0.0080
140°C	0.0065
190°C	0.0069
240°C	0.0081

Table 6	
Sample	**Area**
neat	0.0073
120°C	0.0054
170°C	0.0060
220°C	0.0067
230°C	0.0059

Table 7	
Sample	**Area**
neat	0.0072
120°C	0.0071
170°C	0.0062
220°C	0.0064

Unlike canola oil, spectra of unheated neat samples from the remaining vegetable oils showed no initial *trans* band, shown in Figures 15-17. Spectra shape shifted with increasing

temperature in the same way as canola oil for the remaining oils. Nevertheless, temperatures near the smoke point produced spectra that had an easily identifiable *trans* band, also shown in Figures 15-17.

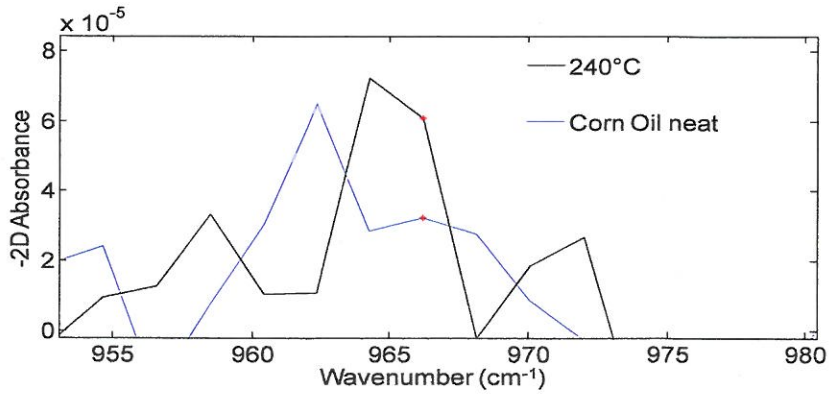

Figure 15-*2D spectrum of corn oil neat versus the most extreme heated sample (240 °C)*

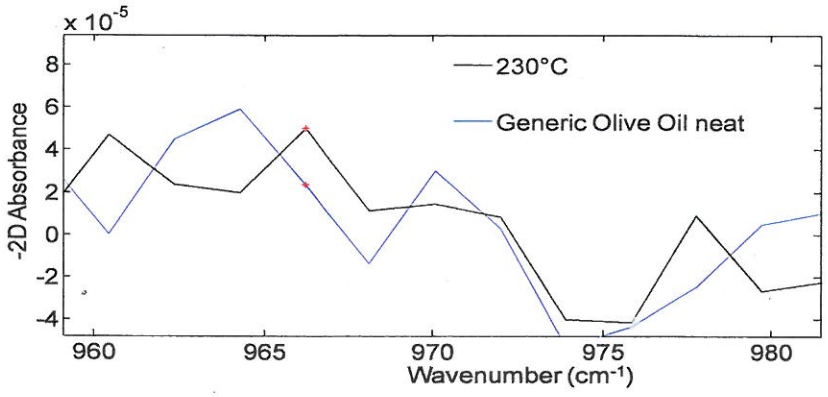

Figure 16 -*2D spectrum of generic olive oil neat versus the most extreme heated sample (230 °C)*

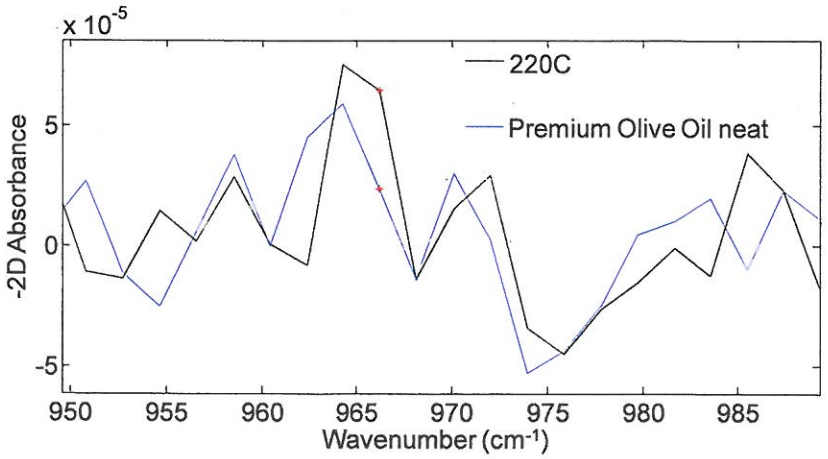

Figure 17 -*2D spectrum of premium olive oil neat versus the most extreme heated sample (220 °C)*

Oleic Acid

Spectra from oleic acid samples, seen in Figure 18, showed a *trans* band that was clearly defined and easily distinguishable, much like spectra from the calibration standards. This is due to the fact that there were no saturated fatty acids in the samples to interfere with *trans* absorption.

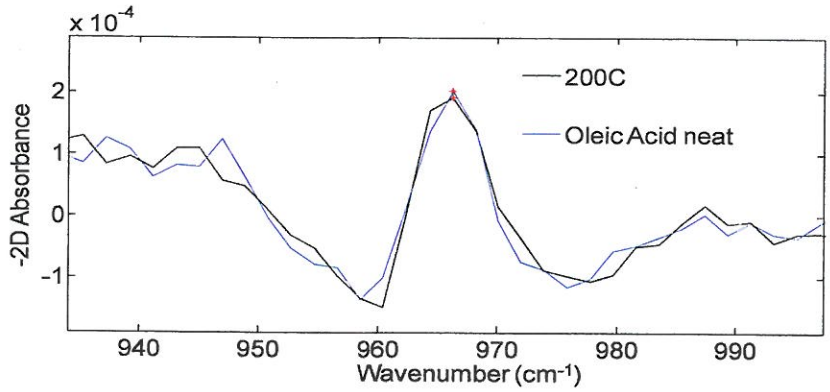

Figure 18 *-2D spectrum of oleic acid neat versus the most extreme heated sample (200 °C)*

Nevertheless, band areas fluctuated slightly within a given temperature and no correlation was seen between area and increasing time. Once again, the areas for all samples within a given temperature were averaged together and are listed below in Tables 8 in comparison to the *trans* band area of the neat oleic acid. Area of the *trans* band in 100°C and 150°C samples were less than the area of neat oleic acid, while the area of the 200°C was slightly larger than neat oleic acid. However, this is indicative of very little, considering how identical the spectra of neat oleic acid and the 200°C sample are, as seen in Figure 18. Differences in area can thus be attributed to minor fluctuations in band shape that occur inherently within analyzing multiple samples.

Table 8 *Area values for* trans *band in oleic acid samples*

Sample	Area
neat	0.0152
100°C	0.0148
150°C	0.0135
200°C	0.0163

GC-MS Results

The GC-MS method used here did not elicit the desired chromatogram.[4,10,14,19] Instead of a clearly isolated peak for every individual fatty acid, the peaks overlapped and had long tails as they eluted, as seen in Figure 19. The sample that was injected was perhaps too concentrated and the peak splitting observed in Figure 19 could then be attributed to column overload. Furthermore, the all purpose VF-1ms column used was not prepped before analysis. The first two feet of the column were not cut off to prevent contamination and, because of this omitted caution, such possible contamination could have also contributed to the poor chromatogram results.

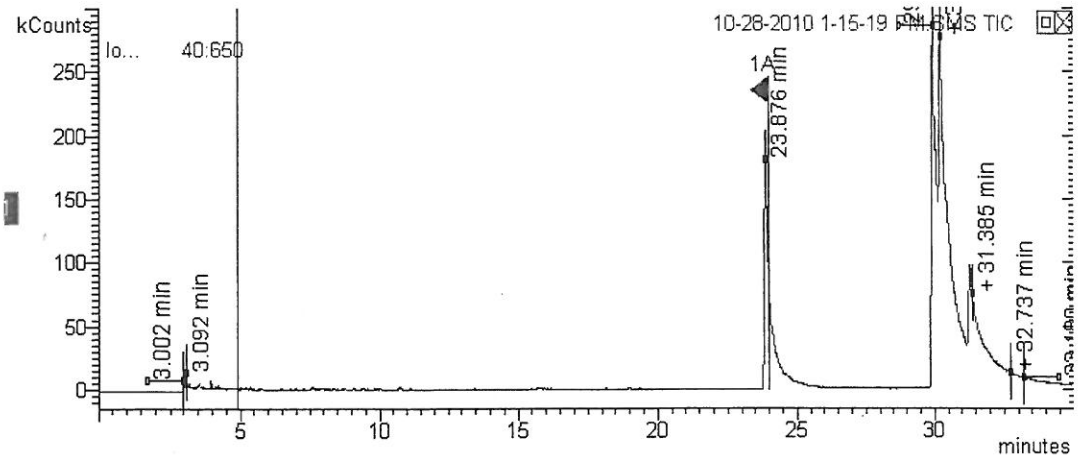

Figure 19 *Spectrum of Crisco from the GC-MS*

All peaks in the chromatogram were identified by the mass spectrum to be fatty acid methyl esters. The first large peak, starting at around 24 minutes, was flagged and the identification data from the MS is shown in Figure 20.

Match 1 of 14 for Scan: 1522 (23.964 mi

Or...	Name	R...	F...	Pr...	MW	CAS No.	Formula
1	Pentadecanoic acid, 1...	859	859	42...	270	5129-60-2	C17H34O2
2	Pentadecanoic acid, 1...	847	847	28...	270	5487-50-3	C17H34O2
3	Hexadecanoic acid, m...	851	838	20...	270	112-39-0	C17H34O2
4	Hexadecanoic acid, m...	834	822	20...	270	112-39-0	C17H34O2
5	Hexadecanoic acid, 2-...	804	799	4...	270	27147-71-3	C17H34O2
6	Pentadecanoic acid, 1...	808	797	42...	270	5129-60-2	C17H34O2
7	Hexadecanoic acid, m...	794	790	20...	270	112-39-0	C17H34O2
8	Hexadecanoic acid, m...	794	782	20...	270	112-39-0	C17H34O2
9	Hexadecanoic acid, m...	787	773	20...	270	112-39-0	C17H34O2
10	Hexadecanoic acid, m...	758	748	20...	270	112-39-0	C17H34O2
11	Octadecanoic acid, 3-...	747	747	0...	510	17369-87-8	C33H66O3
12	Pentadecanoic acid, 1...	757	746	42...	270	5129-60-2	C17H34O2
13	Octadecanoic acid, m...	726	713	0...	298	112-61-8	C19H38O2
14	Octadecanoic acid, 17...	706	706	0...	312	55124-97-5	C20H40O2

Figure 20 *Screenshot of table from MS programming that identified the compounds in the spectrum's first peak*

Nevertheless, the results from the GC-MS further emphasize the advantages of ATR-FTIR over GC when it comes to analyzing *trans* fat content. The ATR-FTIR, without any special modification, was able to assess *trans* content from standards and oil samples, that required no preparation, in about 5 minutes per analysis. With the GC-MS, each sample took between 30 and 35 min to pass through the column. Every sample for analysis had to be extracted and converted to methyl esters before it could be used in the GC. Furthermore, for a desirable spectrum to be obtained, the proper column would have to be purchased and properly installed in the GC oven. The above limitations of the GC-MS alone, without further complications from analyzing quantitatively, would have made it a very cumbersome, time consuming, and expensive method use in order to analyze the *trans* fat content in the vegetable oil samples of this experiment.

Conclusion

The negative second derivative method successfully resolved the infrared *trans* absorption band at 966 cm^{-1} for all *trans* standards. A functional calibration curve was constructed using the area of the -2D *trans* band from each *trans* standard. However, the area of the *trans* bands for all samples of heated vegetable oil and heated oleic acid were below the calibration range (0.5 – 40% *trans* fat). Furthermore, the *trans* peak from heated oil samples was not strong enough to overcome interferences created by saturated fat absorptions. This led to fluctuating baselines and inconsistent shapes for the *trans* band. Despite the above, a qualitative analysis using GC-MS emphasized the GC method's limitations and ATR-FTIR method's advantages for multiple and rapid determination of *trans* fat content.

The results indicate that heating canola, corn, and premium and generic olive oil to their smoke point for at least 15 minutes does not quantifiably increase their respective concentration of *trans* fatty acids. Those who are inclined to cook with a vegetable oil medium, to avoid the saturated fats of butter or margarine and to have the health benefits from the oil's polyunsaturated fats, now have further justification for their choice of cooking medium.

Results could have been improved by using a data analysis program that was equipped with a pre-existing and more accurate integration function. The trapezoid rule, while reliable, is nonetheless an approximation method for integration. Future work could also include heating oil samples to higher temperatures and for longer durations to assess when significant quantities of heat induced *cis/trans* isomerization does occur. Results from this would perhaps not be as relevant to human health or cooking but would provide interesting data.

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