

# Rapid Determination of *Trans* Fats in Thermally Oxidized Soybean Oil by Transmission FT-IR Spectroscopy

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**Abstract** The oxidative deterioration of soybean oil during intermittent heating associated with 190 °C was monitored by transmission Fourier transform infrared spectroscopy in conjunction with partial least square approach. Transmission infrared spectroscopy was applied to attain better sensitivity using 200 μm KCl cell. The oxidation of unsaturated fatty acids, due to this, the formation of *trans* fatty acids occurred during heating at different interval of times from fresh to 12 hour at constant temperature of 190 °C. The best regression at 0.999 was obtained for the determination of *trans* fats and there is an increased pattern of *trans* fatty acids in thermally oxidized soybean oil at temperature of 190 °C. The result of the study shows that transmission infrared spectroscopy can be used as a fast and accurate method for the determination of *trans* fatty acids in the heated edible oils.

**Keywords:** *trans* fatty acids, soybean oil, thermal oxidation, Transmission FT-IR spectroscopy

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## 1. Introduction

Edible oils are an important part of the human diet and more than 90% of global production is used as food or as ingredients in food products. Oils in the diet are a rich source of energy, they contain certain fatty acids, which are nutritionally indispensable and their functional and textural characteristics add to the flavor and acceptability of many natural and processed foods.

Oils begin to decompose from the moment they are isolated from their natural environment. Changes occur causing a disagreeable taste and smell. Atmospheric oxidation is the most important cause of deterioration in oils. This oxidative rancidity is accelerated by exposure to heat, light, humidity and the presence of trace transition metals [3]. The intense frying or heating of oil causes an oxidizing thermal degradation with the formation of decomposition products and change in physical properties [1,2].

The fatty acids are playing the major role on oxidation especially polyunsaturated fatty acids (PUFAs). The

PUFAs which cannot be synthesized by the human body must be present in the diet to avoid symptoms caused by shortage. PUFAs contained as linoleic and linolenic acids specifically in the edible oils and they are necessary for the normal development and functioning of human tissues and are known as essential fatty acids [1].

The interest in *trans* fatty acids (TFAs) has increased in the past decades, because of the relation between TFAs intake and the risk of cardiovascular disease [4], and could also be associated with chronic respiratory disease, neural degenerative diseases and cancer [5].

Spectroscopic methods can be employed to evaluate deterioration in oils subjected to intense heat [6]. Transmission Fourier transform infrared (FT-IR) spectroscopy provides a quick and accurate way of evaluating thermal degradation or heating in these lipids. FT-IR has been used to determine the many deterioration or quality parameters of edible oils [7-12].

This paper reports a methodology to evaluate the variation in composition of soybean oil when it is subjected to intense heat at 220 °C using Transmission FT-IR Spectroscopy. There is a need to determine the TFAs content in the heated or fried oils for a rapid means of

evaluating the nutritive value of oil. The Transmission FT-IR in conjunction with partial least square (PLS) using 200  $\mu\text{m}$  KCl cell has better sensitivity due to greater effective path length.

The objective of this study was to determine whether transmission infrared spectroscopy could serve as an accurate and rapid procedure to determine the total TFAs content in thermally oxidized soybean oil.

## 2. Experimental

### 2.1. Samples and Reagents

All reagents used were of analytical grade and purchased from E. Merck (Darmstadt, Germany). Refined, bleached, and deodorized (RBD) soybean oil was obtained locally from industry suppliers.

### 2.2. Procedure of Thermal Oxidation

Soybean oil was thermally oxidized in an electric deep fryer (E-2016) Bordeaux, France of 2L capacity, from 1 to 12 hour (h) at constant temperature of 190  $^{\circ}\text{C}$ . After every one h, the oil was left to cool down for 30 min and about 20 mL of thermally oxidized oil samples were taken from fryer. This process continued for 12 h and total twelve oil samples were collected.

### 2.3. Preparation of TFA FT-IR Calibration Standards

A group of 14 standards was prepared by gravimetric addition of trielaidine to trans free refined, bleached and deodorized (RBD) canola oil covering a range of trans fat contents from 0.02% to 5.14%, as reported earlier [12].

### 2.4. Determination of *trans* Fats by Transmission FT-IR Spectroscopy

All infrared spectra were acquired using a Thermo Nicolet Avatar 330 FT-IR spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector and KBr optics and controlled by OMNIC software version 7.1 (Thermo Nicolet Analytical Instruments, Madison, WI)

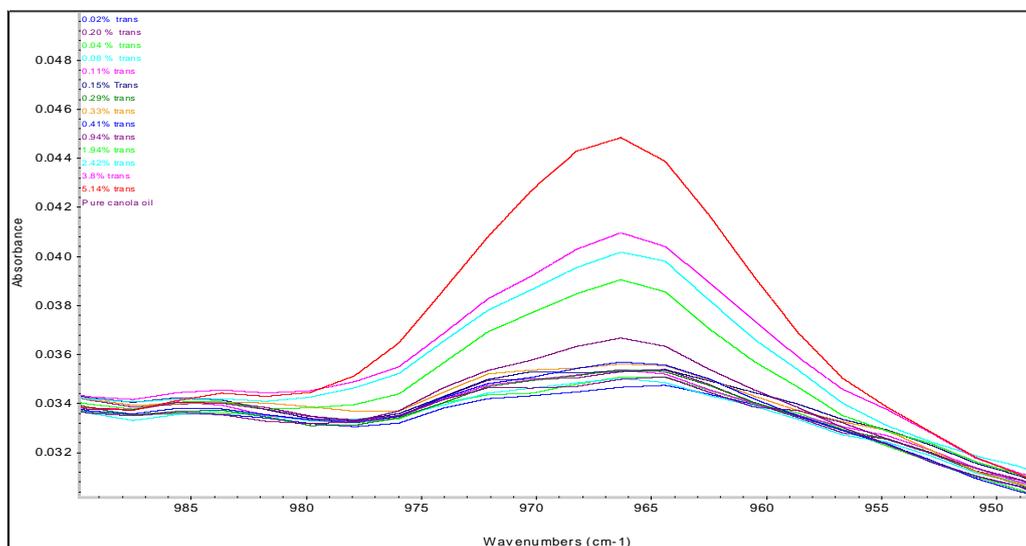
with spectra collected by co-addition of 16 scans at a resolution of 4  $\text{cm}^{-1}$ . The spectrum of each sample was rationed against a fresh background spectrum.

## 2.5. Determination of TFAs by Gas Chromatography-Mass Spectrometry (GC-MS)

For the determination of *trans* fats of fresh and heated soybean oil samples, fatty acid methyl esters (FAMES) were prepared using standard IUPAC method [13]. The GC-MS analysis of FAMES was carried out using an Agilent Technologies gas chromatograph (GC-6890 N, Little Fall, NY, USA) equipped with an Agilent autosampler 7683-B injector and MS-5975 inert XL Mass selective detector. Analytical separation was achieved using Rt-2560 Biscyanopropylsiloxane capillary column (100 m x 0.25 mm i.d x 0.25 *micron* film thickness) for the separation of FAMES. The initial temperature of 140  $^{\circ}\text{C}$  was maintained for 2 min, raised to 230  $^{\circ}\text{C}$  at the rate of 4  $^{\circ}\text{C}/\text{min}$ , and kept at 230  $^{\circ}\text{C}$  for 5 min. The split ratio was 1:50, and helium was used as a carrier gas with the flow rate of 0.8 ml/min. The injector and detector temperatures were 240 and 260  $^{\circ}\text{C}$ , respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV; with an ion source temperature of 230  $^{\circ}\text{C}$ , a quadruple temperature of 150  $^{\circ}\text{C}$ , and a translating line temperature of 270  $^{\circ}\text{C}$ . The mass scan ranged from 50 – 550  $m/z$  with an Em voltage, 1035 V.

Peak identification of the fatty acids in the analyzed fresh and used commercial oil samples were identified by the comparison of their GC retention times with those of reference solutions of the FAMES and also by similarity of their mass spectra with either known fatty acids or published spectra (NIST & Wiley). Standard methyl esters of palmitic, stearic, oleic, elaidic, linoleic, linoelaidic and linolenic acids were used for the confirmation of GC-MS libraries result. Each sample was analyzed triplicate and the data obtained were put into Origin version 7 program and reported as mean ( $n=2 \times 3 \pm$  standard deviation).

## 3. Results and Discussion



**Figure 1.** Overlaid spectra in the *trans* absorption region of calibration standards prepared by addition of various amounts of trielaidin to a *trans* free canola oil ranging from 0.02% to 5.14%.

Figure 1 showing the calibration of *trans* fat was prepared from trielaidin (TE) standards in the range of 0.02 to 5.14%. They were then gently melted and thoroughly mixed before being onto the KBr cell. PLS calibration was performed for the determination of *trans* fats in thermally oxidized soybean oil, Simply run of sample on FT-IR, and then you have analyzed *trans* fats with an assay time of less than 1 min. Groups file of the calibration spectra from OMNIC program was put into TQ Analyst software to get calibration model of TFAs. The region of 970-960  $\text{cm}^{-1}$  provided the satisfactory results in terms of calibration and prediction. The region was selected in TQ software to obtain the best regression.

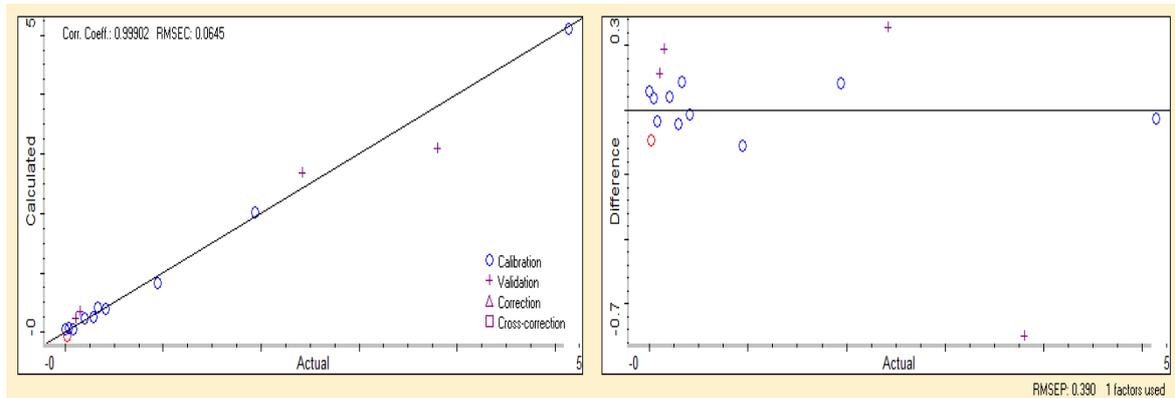
For the partial least square (PLS) model out of fifteen calibration standards, eleven of them were used as a calibration points (o) and four used as a validation points (+). It shows the correlation coefficient of the fraction of the difference in the calculated TFAs values predicted by FT-IR that is accounted by the actual calibration standards. The residual values of TFAs concentration was determined from PLS regression. The highest value ( $R^2 = 0.9990$ ) of the correlation coefficient indicates a highly

linear dependence of the absorption on the concentration of TE, as shown in Figure 2.

**Table 1. Prediction capabilities of PLS-Transmission FT-IR model for the determination of TFAs**

Spectral region	970-960 $\text{cm}^{-1}$
Factors	1
Validation standards	4
R <sup>2</sup>	0.999
RMSEC	0.0645
RMSEP	0.390
RMSECV	0.094

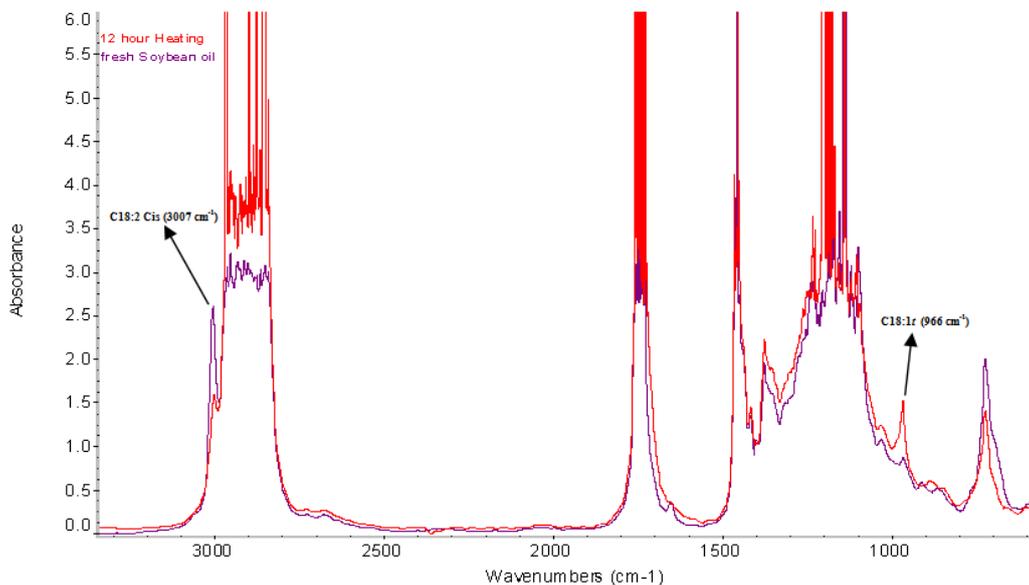
Table 1 shows the other parameters which obtained by PLS calibration model, which have the correlation of coefficient ( $R^2$ ), root mean square error of calibration, prediction and cross validation (RMSEC, RMSEP and RMSECV) and number of factors. The number of factors was automatically selected by the TQ software; it used to attain the lowest possible predicted residual error of sum of squares (PRESS) value. The best results were obtained of  $R^2$ , RMSEC, RMSEP and RMSECV as 0.999, 0.0645, 0.390 and 0.094.



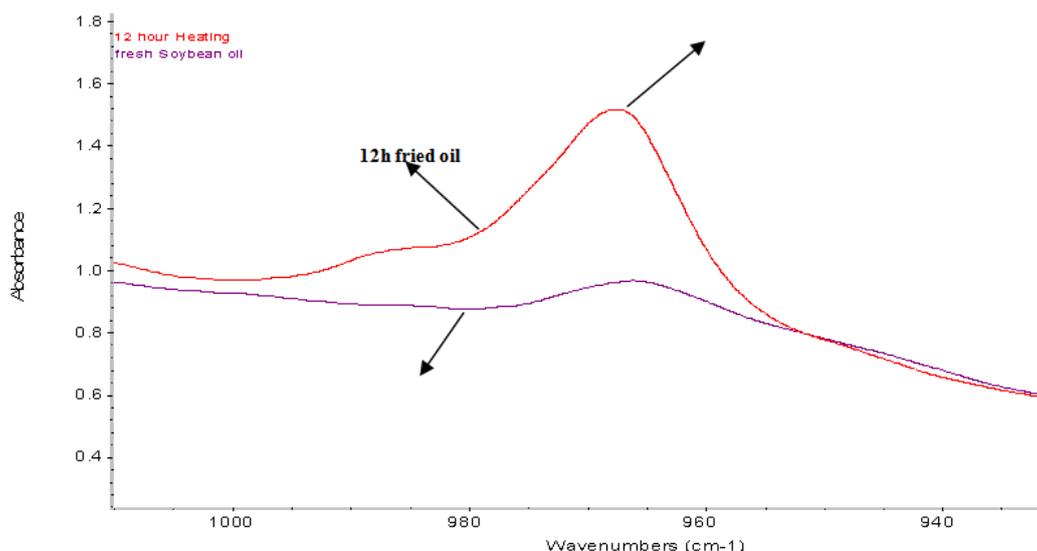
**Figure 2.** Calibration plot derived from the standards spectra by PLS model.

Figure 3 shows the comparison of fresh and heated soybean oil, the intense frying or heating produces a variation in composition of the edible oil nature. The absorption band at around 966  $\text{cm}^{-1}$  is characteristic of isolated *trans* double bonds (C18:1t), and measurement of

this band provides the determination of *trans* content [14]. Cis double bonds (C18:2cis) are characterized by their CH stretching band at 3007  $\text{cm}^{-1}$  [15]. It was clear from the Figure 3 as heating time increases the formation of *trans* fat increases while unsaturation decreases.



**Figure 3.** FT-IR spectra of unheated and heated soybean oil at 190 °C

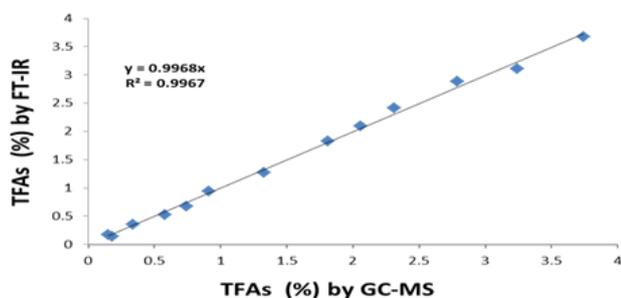


**Figure 4.** Variation in *Trans*'s level of fresh and after 12 h in thermally oxidized soybean oil at constant temperature of 190 °C.

Figure 4. showing the comparative expanded spectra of *trans* fat of fresh and 12 h thermally oxidized soybean oil. However, thermal treatments, such as the frying or heating process, have produced diverse amounts of *trans* fats depending on the oils used.

The formation of TFAs during food frying or heating is closely related to the process temperature and oil use time.

Figure 5 demonstrates the mean transmission FTIR-predicted TFAs versus their mean GC-MS TFAs values. The best regression correlation coefficient of 0.9963 obtained by the linear equation for the plot was  $Y=A+BX$ .



**Figure 5.** Correlation plot of mean GC-MS method versus transmission FT-IR predicted TFAs in thermally oxidized soybean oil

**Table 2.** *Trans*'s fats (%) of thermally oxidized soybean oil at constant temperature of 190 °C by transmission FTIR method

Samples	TFAs (%) GC-MS Method	TFAs (%) FTIR Method
Fresh	0.18±0.01	0.14±0.05
SO-1	0.15±0.01	0.17±0.09
SO-2	0.34±0.04	0.35±0.01
SO-3	0.58±0.07	0.53±0.01
SO-4	0.74±0.01	0.68±0.03
SO-5	0.91±0.05	0.94±0.05
SO-6	1.33±0.10	1.27±0.08
SO-7	1.81±0.12	1.83±0.06
SO-8	2.06±0.06	2.10±0.09
SO-9	2.31±0.08	2.42±0.01
SO-10	2.79±0.08	2.89±0.01
SO-11	3.24±0.01	3.11±0.07
SO-12	3.74±0.03	3.67±0.03

Table 2 presents a comparative result of mean TFAs values acquired from triplicate analyses by GC-MS and transmission FTIR method. No significant difference was observed between the standard reported and transmission

FTIR methods in the values of TFAs for all analyzed samples.

## 4. Conclusions

The results of the present study show that transmission FT-IR spectroscopy and PLS chemometric technique could be applied for the fast determination of *trans* fats in frying soybean oil. Just a sample run on FT-IR, and analyzed the *trans* fats with an assay time of 1-2 min. The transmission FT-IR spectroscopy has been fruitfully applied without using any organic solvent. The transmission FT-IR method is very simple, rapid, environmental friendly and no sample preparation is required as compared to reported standard methods. The best correlation of co-efficient was obtained between reported and standard method as 0.9963. Therefore, proposed method could be effectively applied for the rapid analysis of *trans* fats in the fast food restaurants, edible oil and food frying industries.

FT-IR Spectroscopy provides very useful information on the composition and the extent of thermal self-oxidation in soybean oil subjected to intense heat, equivalent to that used in the ordinary preparation of food. The methodology developed in this study permitted determination of the percentage *trans* fats in the samples at different heating of hours and constant temperature, with no prior treatment. It can be said that when an edible oil is subjected to a heating process or frying, the initial nature of the triglyceride changes significantly and the decrease in unsaturation and increase in *trans* fats is evidence of the transformation occurring in the essential polyunsaturated fatty acids which the oil initially contains, thus indicating a decrease in the oils' nutritional value.

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## References

- [1] Kamal-Eldin, A. and Appelqvist, L.A. (1996). Aldehydic acids in frying oils: formation, toxicological significance and analysis. *Grasas y Aceites*. 47: 342-348.
- [2] Tyagi, V.K. and Vasishtha, A.K. (1996). Changes in characteristics and composition of oils during deep fat frying. *J. of the Am. Oil Chem. Soc.* 73: 499-506.
- [3] Primo Yu'fera, E. (1979). *Quimica Agricola*. Vol. III, Alimentos, Alhambra (Ed.), Madrid, 315-334.
- [4] Dausch, J. G. (2002). *trans*-Fatty acids: A regulatory update. *Journal of the American Dietetic Association*, 102: 18-20.
- [5] Liu, W. H., Inbaraj, B. S. and Chen, B. H. (2007). Analysis and formation of *trans*-fatty acids in hydrogenated soybean oil during heating. *Food Chem.* 104: 1740-1749.
- [6] Engelsen, S.B. (1997). Explorative spectrometric evaluation of frying oil deterioration. *J. of the Am. Oil Chem. Soc.* 74: 1495-1508.
- [7] Van de Voort, F.R. (1994). FTIR Spectroscopy in Edible oil analysis, *Inform* 5 (9): 1038-1042.
- [8] Mahesar, S. A. Sherazi, S. T. H., Kandhro, A. A., Bhangar, M. I., Khaskheli, A. R. and Talpur, M.Y. (2011). Evaluation of important fatty acid ratios in poultry feed lipids by ATR FTIR spectroscopy. *Vib. Spectrosc.* 57 (2): 177-181.
- [9] Mahesar, S. A., Bendini, A., Cerretani, L., Carbognin, M. B. and Sherazi, S.T.H. (2010). Application of a spectroscopic method to estimate the olive oil oxidative status. *Eur. J. Lipid Sci. Technol.* 112: 1356-1362.
- [10] Sherazi, S. T. H., Talpur, M. Y., Mahesar, S. A., Kandhro, A. and Arain, S. (2009). Main fatty acid classes in vegetable oils by SB-ATR-Fourier transform infrared (FTIR) spectroscopy. *Talanta*, 80: 600-606.
- [11] Talpur, M. Y., Sherazi, S. T. H., Mahesar, S. A., Naz, S. and Kara, H. (2012). Impact of frying on key fatty acid ratios of canola oil. *Eur. J. Lipid Sci. Technol.* 114 (2): 222-228.
- [12] Mahesar, S. A., Kandhro Aftab, A., Cerretani, L., Bendini, A., Sherazi, S. T. H. and Bhangar, M. I. (2010). Determination of total *trans* fat content in Pakistani cereal based foods by SB-HATR FT-IR spectroscopy coupled with partial least square regression. *Food Chem.* 123: 1289-1293.
- [13] IUPAC official methods. (1979). 6th ed., *Pergamon Press, Oxford, U. K.*
- [14] Silverstein, R. M., Blaser, G. C. and Morrill, T. C. (1974). *Spectrometric identification of organic compounds*. 3rd ed. John Wiley & Sons, New York.
- [15] Guillen, M. D. and Cabo, N. (1997). Characterization of edible oils and lard by Fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. *J. of the Am. Oil Chem. Soc.* 74 (10): 1281-1286.