Determination of *trans* Fat in Selected Fast Food Products and Hydrogenated Fats of India Using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy

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Abstract: This paper reports the application of a simple and rapid method for the determination of *trans* fatty acid (TFA) content in some of the selected Indian fast food products and hydrogenated fats using Fourier transform infrared (FTIR) spectroscopy in conjunction with second derivative procedure. FTIR spectroscopy has been successfully applied to *trans* measurement using the absorbance bands at or near 966 cm⁻¹ in the FTIR spectra. It was found from the analysis that TFA content of fast food product was ranging from 1.57% to 3.83% of the total fat while for hydrogenated fats, comparatively large quantity of TFA was detected in the range of 3.31% to 4.73%. Since GC-FID is most widely used method for the determination of fatty acid (FA) composition, this method was used for the sake of comparison. Value of regression coefficient was found very close to one (0.99503) with standard deviation of 0.10247 showing a good agreement between GC-FID and proposed ATR-FTIR method.

Key words: *trans* fatty acid, ATR-FTIR, second derivative, fast food, hydrogenated fats

1 Introduction

TFAs intake has a direct relation on human health and they give rise to a number of diseases such as cardiac, cerebral and vascular diseases; vascular inflammation and alteration of blood lipid metabolism. The United State Food and Drug Administration (US FDA) is now taking action to eliminate *trans* fats (TFs) from the food supply because of the highly damaging nature of even very miniscule amount of TFA consumption. On January 1, 2006, the US FDA mandated that it is compulsory to indicate the content of TFAs, under the nutrition facts label of all conventional foods and supplements. This ruling has compelled the food manufacturers to modify their products by decreasing the levels of TFA. It has also increased the awareness about TFAs in the general public and glowed efforts have been taken by a number of countries to limit the TFA content in restaurant foods. In June 2015, US FDA declared that partially hydrogenated oils, which are the primary source of manufactured TFAs, are no longer "generally recognized as safe". The FDA has set a compliance period of three years. This will allow companies to either reformulate products without partially hydrogenated oils (PHOs) or appeal the FDA to allow the specific uses of PHOs.

Official ATR-FTIR methods for the rapid determination of total *trans* fatty acids in fats and oils were adopted as AOCS Cd 14-99, AOAC 2000.10 and AOCS Cd 14e-09, and the latter was applied in the present study. For the accurate determination of fatty acid composition, official GC method AOCS Ce 1h-05 was used. In food products, extracted fats are also analyzed to determine TFA using official methods. FTIR technique is used for the determination of total *trans* fat content (TFC) unlike gas chromatography (GC), which provides detailed information of FA composition of all fat containing products. In GC analysis, samples are converted into fatty acid methyl esters (FAMEs) before analysis. Use of highly polar columns (fused silica capillary columns coated with highly polar cyanopolysiloxane stationary phases) are most suitable for the analysis of *cis* and *trans* isomers. GC analysis has some drawbacks, such as more time consumption and derivatization of extracted fats, which made us to use FTIR procedure for the desired analysis.

After viewing the above mention facts, it becomes an important research task to analyze the TFC in fast foods and...
hydrogenated fats for consumer welfare. Traditional FTIR procedure was having some limitations, such as baseline offset and slope, the need for a trans free or any reference background and spectral overlap. Irrespective of the sampling technique, the baseline shifts and variations in background absorptions in the spectral profiles, affect quantitative determinations. These shortcomings were found to no longer exist with second derivative treatment. The ATR-FTIR procedure has been used for the determination of total TFC, by measuring the height of the negative second derivative. A mixture of glycerides of TFAs and conjugated linoleic acid (CLA) were first analyzed using second derivative reflectance spectrum in virgin oil by FTIR. The narrower width of a second derivative band, relative to that of the absorption band of FTIR spectra, helped to quantify the total TFC very rapidly and more accurate than other applied methods. This negative second derivative method demonstrated to be the most appropriate for the rapid determination of total TF for nutrition labeling. Negative second derivative spectra was used to make peaks of second derivative spectra in upside direction and it was obtained by multiplying second derivative data to -1. In the present study, we evaluated the TFA in 8 fat samples extracted from fast food products and 7 different partially hydrogenated fats (PHOx, where x = 1-7) were also purchased from different markets of Aligarh, India. All reagents and solvents were of analytical grade and purchased from Sd Fine Ltd (India). Pure standards of FAMEs, triolein and trielaidin were purchased from Sigma Aldrich.

### 2 Experimental procedures

#### 2.1 Sampling, reagents and standards

Eight types of fast food products such as a variety of chips and biscuits, microwave popcorn, pastry and namkeen (wafer) were collected from market and seven different partially hydrogenated fats (PHOx, where x = 1-7) were first analyzed using second derivative reflectance spectrum in virgin oil by FTIR. The narrow width of a second derivative band, relative to that of the absorption band of FTIR spectra, helped to quantify the total TFC very rapidly (<5 min) and more accurate than other applied methods. This negative second derivative method demonstrated to be the most appropriate for the rapid determination of total TFA for nutrition labeling verification. Negative second derivative spectra was used to make peaks of second derivative spectra in upside direction and it was obtained by multiplying second derivative data to -1. In the present study, we evaluated the TFA in 8 fat samples extracted from fast food products and 7 different hydrogenated fat samples purchased from local markets of Aligarh, India. We have used ATR-FTIR with the support of GC-FID for the desired analysis. Some of the fast food products were labeled as containing zero trans fat, but found to have an appreciable amount of TFA after completion of analysis. The predicted percent TFA in fast food product was ranging from 1.57% to 2.83%, while higher level of TFA was found in hydrogenated fat samples ranging from 3.31% to 4.73% (Table 1).

#### 2.2 Chromatographic techniques

Thin layer chromatography (TLC) was made on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai, India, 0.5-mm thickness). The spots of the compounds were observed on exposure to iodine vapor. A mixture of petroleum ether-diethyl ether-acetic acid (7:3:1, v/v) were used as developing solvents. Hexane-diethyl ether (94:6, v/v) was used as eluent for column chromatography.

### Table 1 Prediction of TFA content in 15 samples by ATR-FTIR and GC-FID methods.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sample</th>
<th>Total fat (g/100g sample) Mean ± SD</th>
<th>Predicted %TFA to the total fat</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>*</td>
<td>By ATR-FTIR</td>
<td>By GC-FID</td>
</tr>
<tr>
<td>1</td>
<td>Potato chip</td>
<td>16.53 ± 1.27</td>
<td>2.65</td>
<td>2.61</td>
</tr>
<tr>
<td>2</td>
<td>Biscuit 1</td>
<td>15.29 ± 2.41</td>
<td>1.81</td>
<td>1.69</td>
</tr>
<tr>
<td>3</td>
<td>Popcorn</td>
<td>19.48 ± 1.53</td>
<td>1.57</td>
<td>1.51</td>
</tr>
<tr>
<td>4</td>
<td>Biscuit 2</td>
<td>15.91 ± 1.72</td>
<td>3.83</td>
<td>3.73</td>
</tr>
<tr>
<td>5</td>
<td>Pastry</td>
<td>20.53 ± 2.21</td>
<td>2.63</td>
<td>2.69</td>
</tr>
<tr>
<td>6</td>
<td>Biscuit 3</td>
<td>18.42 ± 2.48</td>
<td>2.01</td>
<td>2.12</td>
</tr>
<tr>
<td>7</td>
<td>Banana Chip</td>
<td>17.63 ± 2.03</td>
<td>2.19</td>
<td>2.09</td>
</tr>
<tr>
<td>8</td>
<td>Namkeen</td>
<td>15.74 ± 1.65</td>
<td>2.49</td>
<td>2.55</td>
</tr>
<tr>
<td>9</td>
<td>**PHO1</td>
<td>-</td>
<td>3.58</td>
<td>3.56</td>
</tr>
<tr>
<td>10</td>
<td>**PHO2</td>
<td>-</td>
<td>3.93</td>
<td>4.06</td>
</tr>
<tr>
<td>11</td>
<td>**PHO3</td>
<td>-</td>
<td>4.73</td>
<td>4.56</td>
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<tr>
<td>12</td>
<td>**PHO4</td>
<td>-</td>
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<td>3.45</td>
</tr>
<tr>
<td>13</td>
<td>**PHO5</td>
<td>-</td>
<td>4.10</td>
<td>4.03</td>
</tr>
<tr>
<td>14</td>
<td>**PHO6</td>
<td>-</td>
<td>4.57</td>
<td>4.48</td>
</tr>
<tr>
<td>15</td>
<td>**PHO7</td>
<td>-</td>
<td>3.36</td>
<td>3.24</td>
</tr>
</tbody>
</table>

*All values are mean of triplicate extraction along with standard deviation.

**All PHOx (x = 1-7) samples were hydrogenated fats.
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2.3 Extraction of Oil

Total lipids were extracted from fast food samples according to AOAC official method 996.06 with slight modifications. Test food samples were crushed and transferred into 250 mL RB. A mixture of chloroform (15 mL), ethanol (15 mL) and 8.3 M HCl (75 mL) were added to the RB and heated at 80°C for 1 h. The contents of the RB were poured into 250 mL beaker through glass funnel lined with Whatman filter paper. The solvent was removed under reduced pressure. The residue was dissolved in diethyl ether and water (50:30, v/v) in a separatory funnel to get total oil content in the organic layer. The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated.

2.4 Preparation of FAME

Preparation of FAME derivatives is by far the most common chemical reaction performed by lipid analysts. Following the procedure of AOCS Ce 2-66, briefly, which involved acid hydrolysis of extracted lipids (0.20 g) of each sample with 5% HCl/methanol followed by BF₃ solution. The progress of the reaction was monitored by TLC. After completion, the solvent was removed under pressure and the residue was mixed with diethyl ether/water (30:15, v/v) in a separatory funnel to get a clear organic layer. The separated organic layer containing FAME was passed over anhydrous Na₂SO₄ and solvent was removed under reduced pressure to get yellow oily liquid. The isolated FAMEs were purified by column chromatography using the mixture of n-Hexane-diethyl ether (94:6, v/v) as eluent.

2.5 ATR-FTIR Instrumentation

Absorbance spectra of calibration standards and edible oils were obtained using a Perkin–Elmer Spectrum One FTIR spectrometer (UK) fitted with an Attenuated Total Reflectance (ATR) crystal of zinc selenide. Samples were put on ATR crystal maintained at 65°C to make the crystal completely covered by the sample. A very small amount (50-100 μL) of the sample was needed to cover the surface area of the ATR crystal. The samples were measured in duplicate. The spectra were collected continuously over a wavelength range of 800–4000 cm⁻¹ with a data resolution of 4 cm⁻¹ and air was used as a reference background material. The ATR crystal was cleaned after each scan. Cleaning process of ATR crystal involved three steps. 1) Removal of the sample from the surface of the ATR crystal, 2) Cleaning of the ATR crystal and 3) Drying of the ATR crystal. A tissue paper was used in all the three steps.

2.6 Gas Chromatographic (GC-FID) Analysis

AOCS Official Methods Ce 1h-05 and Ce 1j-07 are the GC methods recommend the use of 100-m cyanopropyl polysiloxane columns, for example the SP-2560 or CP-Sil 88 (Chrompack, Middleburg, The Netherlands). GC-2010 chromatograph (Shimadzu, Japan) fitted with a CP-SIL column (100 m × 0.25 mm × 0.2 nm) and flame ionization detector was used for desired GC analysis. Cross linked siloxane polymer was used as stationary phase and nitrogen as carrier gas. The temperature programming process included initial temperature of 80°C holding for 2 min and increased to 255°C and maintained for 10 min. GC Solution software was used for recording chromatogram.

2.7 Data processing and Calibration

It is evident from the previous studies that the multivariate calibration approach combined with FTIR spectroscopy may improve the accuracy of determination of low TF levels. To enhance spectral features, the second derivatives of absorption spectra were generated. Second derivative method was used for measured ATR-FTIR spectra with second order polynomial. For each sample, peak height at approximately 966 cm⁻¹, which is the characteristic peak of isolated trans double bond, was evaluated in second derivative spectrum using Advance Origin 6.0 software. The pictures changed in most of the cases when the spectral profiles were doubling derivate. This process not only removed the base line effect from the spectral profiles, but also identifies some hidden absorptions bands in the broad IR spectral profiles. A series of standard samples were prepared adopting the reported standard procedure. A varying amounts of trielaidin were mixed with trans free triolein to prepare artificial samples covering the concentration range from 0.2% to 22.0% of TFAs (supplementary file Table 1S).

2.8 Validation

Validation samples were also prepared by adding trielaidin in trans free triolein covering the TFA concentration range from 0.2 to 20%. Measured heights of second derivative spectra of validation samples were substituted as x in the regression equation obtained from calibration curve to determine TFA content (y). Results obtained were compared with gravimetrically determined TFA content.

3 Results and Discussions

3.1 Calibration

As reported in literature, the calibration standards were analyzed for total TFC by measuring the height of the second derivative or the area of the absorbance band at 966 cm⁻¹. In present study, calibration model, including varying concentration of TFA, provided the relationship between calculated value of TFA and height of second derivative spectra (shown in supplementary file Table 1S). This resulted the linear regression plot with the fol-
following equation:

\[ y = 0.0336552x + 0.150121 \]  

(1)

where \( y \) is percent TFA and \( x \) is the height of second derivative spectra at approximately 966 cm\(^{-1}\). Standard deviation and square of regression coefficient in this equation were 0.0428 and 0.9759 respectively. This equation was used for the calculation of TFA of unknown samples by substituting the second derivative peak height of blind samples of their ATR-FTIR spectra in equation (1).

### 3.2 Validation of standard and blind sample

To check the validity of ATR-FTIR predicted results, obtained findings were compared with calculated TFA percentages. A good agreement had been found between calculated and predicted results by applying paired t-Test statistics (supplementary file Table 2S) at 0.05 population level. Obtained t and p value clearly showed that the difference of mean \( t \)ra values obtained by the applied ATR-FTIR method and those obtained by the gravimetric calculation, were statistically not significant at \( p = 0.05 \). Correlation coefficient using regression plot (Fig. 2A) for this comparative study was very close to 1 (\( R = 0.9999 \) with SD of 0.10) (supplementary file Table 3S). A comparative bar graph was also plotted (Fig. 2B) to see the nearness of the results. Blind sample validation study included selected eight fast food products and seven partially hydrogenated fat samples from market. These samples were first analyzed by GC-FID method and then ATR-FTIR to know the difference or similarities between them.

### 3.3 FTIR spectra of fast food and hydrogenated fats

Absorption spectra for calibration standard, using a varying concentration of trielaidin spiked in \( \text{trans} \) free triolein, was obtained (Fig. 3A). A second derivative spectrum was also electronically generated for the same (Fig. 3B). An absorption band near 966 cm\(^{-1}\) was shown by each of the samples which were the result of isolated \( \text{trans} \) band. Visibility of these \( \text{trans} \) bands depended on the amount of TFA and also on the cleanliness of ATR crystal. Intensity of the peak at 966 cm\(^{-1}\) directly depends on the concentration of TFA present in the sample. In some of the fast food products, low intensity \( \text{trans} \) bands were not clearly visible in ATR-FTIR spectra but became evident in the second derivative spectra. This second derivative treatment is specifically applied to the data with high signal to noise ratio because from the earlier studies it is clear that use of second derivative reduces the signal to noise ratio\(^{20}\). Inten-
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J. Oleo Sci. 66, (3) 251-257 (2017)

The density of this peak was slightly high in hydrogenated fats as compared to oils used for frying fast food products. Of the fifteen test samples analyzed, eight samples contained more than 3 percent TFA to the total fat (Table 1). Predicted TFA results for fast food products were found approximately similar to the previously reported findings (26). It was seen by linear regression equation with regression coefficient close to unity (0.9950) (supplementary file Table 4S and Fig. 4A), and paired t-Test statistics (Table 2) that the proposed ATR-FTIR method had an excellent covenant with the GC-FID method. A comparative bar graph was also obtained (Fig. 4B).

3.4 GC-FID analysis

To identify the presence of trans fat content, various mixtures of FAMEs standard were run. Mixture of trielaidin and triolein (standard trans and cis 18:1 FAMEs) was used mainly for evaluation of TFA because despite other trans peaks (18:2 and 18:3), most of the samples were found to have elaidic acid content shown by their GC chromatogram as also reported in earlier studies (27). Separation of these FAMEs was performed adopting the official AOCS method (6). Due to the use of cross linked polysiloxane stationary phase, 18:1 trans isomer (trielaidin) was eluted earlier than its corresponding cis isomer (triolen) (supplementary file Fig. 1S). Trans peaks other than 18:1, was eluted in a group because of integration of 18:2 and 18:3 peaks. Neglecting these group peaks, evaluation of 18:1 TFA peaks in chromatogram for analyzed samples was done by comparing their peaks with a standard mixture of

Fig. 3 (A) ATR-FTIR absorption spectra obtained for varying amount of trielaidin spiked in trans-free triolein; (B) corresponding second derivative spectra.

Fig. 4 (A) Linear regression plot of ATR-FTIR and GC-FID results; (B) comparison of GC-FID and ATR-FTIR results.
corresponding FAMEs. The quantification of elaidic acid was done by calculating peak areas of respective peaks found in trielaidin region.

### Conclusion
In the present study, trans fat content in some of the selected Indian fast foods and commonly used hydrogenated fats was investigated using ATR-FTIR incorporated with second derivative analysis with the support of GC-FID. Fast food products and hydrogenated fats analyzed were found to have 1.57-3.83 trans fatty acid content to the total fat respectively. Among the eight fast food products analyzed, lowest TFA content (1.57%) was found in popcorn oil and highest (3.83%) was in biscuit 2. Hydrogenated fats analyzed were found to have a relatively higher concentration of TFA than fast food extracted oils. Lower TFA containing hydrogenated fats was PHO 4 containing 3.31% TFA and higher one was PHO 3 with 4.73% TFA. Results obtained using ATR-FTIR method was found in good agreement with other previously reported findings. As TFA is one of the main causes of coronary heart diseases, it should be taken off from the local available foods as well as branded food products. To the best of our knowledge, no study has been reported to analyze TFA content in the above mention fast food products and hydrogenated fats in India. Further a study covering larger samples drawn from different grade oils of India should be done.

### Acknowledgement
The author would like to thank Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh, for providing necessary facilities to complete this article.

### Supporting Information
This material is available free of charge via the Internet at http://dx.doi.org/jos.64.10.5650/jos.e59.16168

### Table 2 Statistical analysis of ATR-FTIR and GC-FID results.

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>*Mean of relative differences</th>
<th>Standard deviation</th>
<th>Variance</th>
<th>**t-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>-0.0333</td>
<td>0.1025</td>
<td>0.0105</td>
<td>-0.0712</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*At the 0.05 significance level, the mean of differences was not statistically significant.
** t-value with 15 degrees of freedom.

### References
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