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Analytical Methods

Analysis of trans fatty acids in deep frying oils by three different approaches

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ABSTRACT

Simulated frying experiments were performed on three different types of oils with French fries as the fried food. Comparison of frying oil samples was then made with their control counterparts (i.e. oil samples heated without food). Three different methods, gas chromatography (GC) attenuated total reflection (ATR) AOCS method Cd 14d-99 and attenuated total reflection negative second derivative absorbance (−2D ATR), were applied to quantify total trans fats. The total trans fats were found to be higher in the frying oil samples as compared to the control samples, which might be due to the presence of a high amount of trans fats in the pre-fried and frozen French fries. In general, the ATR AOCS method Cd 14d-99 produced lower amounts of trans fatty acids and the −2D ATR absorbance method produced higher amounts when compared with those obtained by gas chromatography.

1. Introduction

A possible association has been shown between the intake of trans fatty acids and the risk of coronary heart disease. They are proven to produce adverse effects on blood lipids, including increasing LDL-cholestrol concentration and decreasing HDL-cholestrol concentration (Khor & Mohd Esa, 2008). Dietary trans fats not only include mono- and poly unsaturated fatty acids, but also conjugated linoleic acids (Sebedio & Ratnayake, 2008). Partially hydrogenated oils are considered to be the major source of trans fats in the diet. Vegetable oils are partially hydrogenated in the presence of a metal catalyst and hydrogen to produce a more stable product. As a result of this process, the double bonds in unsaturated fatty acids are reduced and some of them are converted from their natural cis to trans configuration (Khor & Mohd Esa, 2008). Other processes, which can lead to the formation of trans isomers include thermal refining, bleaching and deodorization. Amongst common unsaturated fatty acids, linolenic acid is primarily considered responsible for the production of trans fatty acids in thermally induced isomerisation (Grandgirard, Sebedio, & Fluery, 1984).

Deep fat frying has also been considered a source for the production of trans fatty acids. Formation of trans fatty acids during frying has been shown to be closely related to process temperature and time. However, results in the literature have been inconsistent. In sunflower oil, the amount of trans isomers were found to be 1.10% when heated at 200 °C for 40 min as compared to 11.45% at 300 °C for the same duration of heating (Moreno, Olivares, Lopez, Adelantado, & Reig, 1999). However, Romero, Cuesta, and Sánchez-Muniz (2000) reported a very minimal production of elaidic acid in extra virgin olive oil, high oleic sunflower oil and sunflower oil.

Various approaches have been proposed in the literature to quantify isolated trans fats. One of them is the AOCS Official Method Cd 14-95 (AOCS, 1999). The method is based on the CH out of plane deformation band produced at 966 cm^{-1}, a unique feature of the infrared spectra in the presence of isolated trans double bonds in trans-monoenes, trans-trans-dienes, mono-trans-dienes and mono-trans-trienes (Mossoba, Milosevic, Milosevic, Kramer, & Azizian, 2007). The method uses Fourier transform infrared (FTIR) spectroscopy and requires the conversion of oil samples into their fatty acid methyl esters (FAMEs) prior to analysis. As per the previous reports, this approach to quantify isolated trans fats was not considered satisfactory, as it resulted in a strong sloping baseline because of the absorption overlapping with other bands of spectra in FAMEs (Adam, Mossoba, & Lee, 2000; Ali, Angyal, Weaver, Rader, & Mossoba, 1996). The method, which provided relatively higher amounts of trans fats as compared to the results obtained by GC was considered unreliable, especially in samples containing below 5% trans fats (Mossoba et al., 2007). To overcome these problems, another approach using attenuated total reflection (ATR) infrared spectroscopy was proposed (Mossoba, Yurawecz, &

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McDonald, 1996), which was later listed as AOCS Official Method Cd 14d-99 (AOCS, 1999). This method required trans free reference fat having similar fatty acid composition to the samples. For samples of known fatty acid composition, the refined bleached source of the same oil was recommended as the reference fat, whereas for samples of unknown composition, ultra degummed bleached expeller soybean oil was recommended. As per the official method, the minimum limit of quantification was specified as 1.0%. While this method eliminated the sloping baseline problem, finding a reference fat that is free from trans fats and also closely matches the fatty acid composition of a sample is not always possible. A new method was proposed to eliminate the need of trans free reference fat (Milosevic, Milosevic, Kramer, Azizian, & Mossoba, 2004), in which absorption spectrum was produced using air as the background reference material and then the second derivative (2D) of the spectrum was used to quantify trans fat. The 2D spectrum was multiplied by −1 just to get the peaks in the upward direction. By applying the negative second derivative to a spectrum, the resolution of IR bands was enhanced, and it was possible to detect small shifts in IR band position and the presence of other interferences (Mossoba et al., 2007). Currently, the −2D ATR analysis is still a subject of an ongoing AOCS international collaborative validation study.

The most acceptable method to quantify trans fats is gas chromatography coupled with a flame ionization detector (GC-FID). Samples are derivatised to their methyl esters which are then injected to GC. Analysis of cis and trans isomers is best carried out using long, flexible, fused silica capillary columns coated with highly polar cyanopolysiloxane stationary phases containing various polar substituents (Sebedio & Ratnayake, 2008). The most recent proposed method of GC analysis by AOCS is AOCS Official Method Ce 1 h-05 (AOCS, 2005).

The objective of this study was to analyse trans fatty acids in three different frying oils and their changes with increased number of frying cycles during the course of frying frozen French fries in the oils. The three frying oils included palm olein, cooking oil (a blend of palm olein, sesame oil and peanut oil) and sunflower oil.

The palm olein was found to be rich in oleic acid (39.73 ± 0.39%) followed by palmitic acid (34.53 ± 0.71%). A substantial amount of linoleic acid (16.66 ± 1.10%) was also present. However, in the cooking oil, linoleic acid was found to be of higher amount as compared to the palm olein. The addition of peanut oil and sesame oil, both of which are rich in linoleic acid, might have contributed to the higher amount of linoleic acid in the cooking oil. For palm olein, a range of 36.8–43.2% of palmitic acid and 39.8–44.6% of oleic acid has been reported (Rossell, 2001). No such information on the fatty acid composition for cooking oil could be found in the literature. In the sunflower oil, linoleic acid (67.66 ± 1.03%) and oleic acid (19.68 ± 0.82%) were the major fatty acids (Table 1). A range of 48.3–74.0% of linoleic acid and 14.0–39.4% of oleic acid has been reported previously for sunflower oil (Rossell, 2001). Romero et al. (2000) reported a similar fatty acid composition for sunflower oil, including 61.9 ± 0.05% of linoleic acid, 23.6 ± 0.04% of oleic acid and 6.8 ± 0.03% of palmitic acid.

In this study, production of trans fats in heated oil samples without food (control samples) using the same frying protocol was also analysed. Various approaches including ATR AOCS, −2D ATR and GC-FID methods were applied to quantify trans fats.

Table 1
Fatty acid profiles of palm olein (PO), cooking oil (CO) and sunflower oil (SO) before deep frying.

Fatty acid	PO	CO	SO
12:0	0.20 ± 0.02	0.20 ± 0.01	0.05 ± 0.00
14:0	0.89 ± 0.02	1.00 ± 0.11	0.09 ± 0.01
16:0	34.5 ± 0.71	33.1 ± 1.12	6.30 ± 0.22
16:1 cis	0.14 ± 0.02	0.14 ± 0.01	0.00 ± 0.00
18:0	4.05 ± 0.05	3.98 ± 0.14	3.34 ± 0.12
18:1 cis	39.7 ± 0.39	39.6 ± 1.14	19.7 ± 0.82
18:1 trans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:2 cis	16.7 ± 1.10	18.8 ± 0.65	67.7 ± 1.03
18:2 trans	0.31 ± 0.01	0.31 ± 0.02	1.27 ± 0.05
18:3 *n*−4	0.09 ± 0.01	0.12 ± 0.02	0.00 ± 0.00
18:3 *n*−3	0.30 ± 0.06	0.41 ± 0.05	0.24 ± 0.03
20:0	0.84 ± 0.03	1.00 ± 0.08	0.23 ± 0.02
20:1	0.14 ± 0.02	0.19 ± 0.01	0.12 ± 0.01
22:0	0.09 ± 0.01	0.23 ± 0.00	0.65 ± 0.09

2. Materials and methods

2.1. Materials for frying

Three different oils, i.e. refined, bleached and deodorized palm olein (PO) (Duck brand, Lam Soon Singapore Pte Ltd.), cooking oil (CO) (Knife brand, Lam Soon Singapore Pte Ltd.) which was a premixed blend of palm olein, sesame oil and peanut oil, and sunflower oil (SO) (Naturel brand, Lam Soon Singapore Pte Ltd) were purchased from a local supermarket in Singapore. French fries (Farmland brand, Ben Food(s) Pte Ltd, Singapore) were selected as the frying foods and were purchased from a local supermarket.

2.2. Frying protocol

An electric deep fat fryer (Model No. DF23S1B, dimensions 370 × 455 × 350 mm, Sinmah Steel Refrigeration Pte Ltd) with 8 l capacity was filled with 5 l of oil for every batch of experiments. At the start of every day, oil was heated up for 1 h at 180–185 °C. After the initial heating up process, the first frying cycle was started. Subsequent frying cycles were performed at an interval of half an hour. During each frying cycle, 100 g of food i.e. French fries (FF) were fried. Each day, the oil was heated for 6 h in total, with 10 frying cycles performed. The oil was used continuously for 4 days without any replenishment. This amounted to a total of 40 frying cycles performed for each batch of oil. Oil samples were collected every 5 frying cycles. The collected samples were filtered through Whatman No. 4 filter paper to remove suspended food particles, flushed with nitrogen and then stored in glass bottles at −20 °C until further tests.

In total, three simulated frying batches were performed for the three different oils respectively. The three batches are denoted as PO-FF (palm olein with French fries), CO-FF (cooking oil with French fries) and SO-FF (sunflower oil with French fries) respectively in the rest of this paper.

2.3. Control samples

Control samples were produced by heating the oils without frying any food using the protocol described above. Three batches were prepared and are denoted as PO (control cooking oil), CO (control cooking oil) and SO (control sunflower oil).

2.4. Fatty acid standards

Various reference standards of FAMEs were run to identify the fatty acid (FA) peaks in chromatograms. K110 FAME mix was purchased from Alltech-applied Science Labs, PA, USA, whereas F.A.M.E. mix C14-C22, Supelco® 37 component FAME Mix, linoleic acid methyl ester isomer mix and linolenic acid methyl ester isomer mix were purchased from Supelco, Bellefonte, PA, USA. K110 FAME mix included the following methyl esters: methyl palmitate, methyl palmitelaidate, methyl palmitoleate, methyl stearate,

methyl elaidate, methyl oleate, methyl linoelaidate and methyl linoleate. FAME mix C14-C22 included linoelaidic acid methyl ester, methyl myristate, methyl palmitate, methyl stearate, methyl behenate, cis-9-oleic methyl ester, methyl arachidate, trans-9-elaidic methyl ester, methyl linolenate and methyl linoleate. Supelco 37 component FAME mix contained 37 different FAMEs including the cis and trans isomers of C18:1 and C18:2. Linoleic acid methyl ester isomer mix comprised of trans-9, trans-12-octadecadienoic acid methyl ester, cis-9, trans-12-octadecadienoic acid methyl ester, trans-9, cis-12-octadecadienoic acid methyl ester, cis-9 and cis-9, cis-12-octadecadienoic acid methyl ester. Linolenic acid methyl ester isomer mix consisted of trans-9, trans-12, trans-15-octadecatrienoic acid methyl ester, trans-9, trans-12, cis-15-octadecatrienoic acid methyl ester, trans-9, cis-12, trans-15-octadecatrienoic acid methyl ester, cis-9, trans-12, trans-15-octadecatrienoic acid methyl ester, cis-9, cis-12, trans-15-octadecatrienoic acid methyl ester, cis-9, trans-12, cis-15-octadecatrienoic acid methyl ester, trans-9, cis-12, cis-15-octadecatrienoic acid methyl ester and cis-9, cis-12, cis-15-octadecatrienoic acid methyl ester. Methyl tridecanoate (98%, GC grade, Sigma–Aldrich Chemical Co, St Louis, MO, USA) was used as an internal standard.

2.5. Preparation of FAMEs

Corresponding FAMEs of the oil samples were prepared as per the method IUPAC 2.301 (IUPAC, 1987). This method was specific for the preparation of FAMEs for oils and fats having an acid value of less than 2. All the oil samples were tested first for the acid value based on the AOCS Official Method 5a-40 for quantifying free fatty acids and their acid value were found to be less than 2. Methylation was achieved with 2 N methanolic potassium hydroxide and the FAMEs were extracted in n-heptane. Methyl tridecanoate (5 mg/ml) was added as the internal standard for GC analysis.

2.6. Gas chromatography (GC) of FAMEs

GC was performed in a CP 3800 Varian Gas Chromatograph fitted with a FID (GC-FID). A highly polar column, BPX 70 (SGE Analytical Science, Australia) was selected for the analysis. The dimensions of the column were 120 m × 0.25 mm × 0.25 μm. The temperature program was as follows: initial temperature, 140 °C (hold for 5 min); temperature rate, 4 °C/min; final temperature, 240 °C for a final holding time of 20 min. The detector and injector port temperatures were maintained at 260 °C. Helium was used as the carrier gas at a flow rate of 1.3 ml/min. Split ratio of 100:1 was used and 1 μl of sample was injected in GC for the analysis. Star Chromatography Workstation Version 6.0 (Varian, USA) was used to integrate the chromatograms.

2.7. FAME quantification

FA identifications were done based on equivalent chain length (ECL) values provided by the manufacturer and retention times obtained by running different standards. Quantification of FA was done by calculating response factors of individual FA isomers. Weight of each fatty acid was expressed as individual triglyceride in test samples, by using the conversion factors for conversion of FAMEs to triglycerides for individual fatty acids. The detailed procedure was described by Ali, Angyal, Weaver, and Rader (1997).

2.8. Attenuated total reflection infrared spectroscopy – AOCS Cd 14d-99

A Perkin–Elmer Spectrum One FTIR spectrometer (UK) fitted with an ATR infrared cell (zinc selenide crystal) was set up with the following parameters: 900–1100 cm^{-1} range, 64 scans were collected at a resolution of 4 cm^{-1}. A series of trans calibration standards were prepared by weighing varying amounts of trielaidate (⩾99%, Sigma–Aldrich Chemical Co, St Louis, MO, USA) in triolein (⩾99%, Sigma–Aldrich Chemical Co, St Louis, MO, USA). Triolein, used as the reference material, was converted to FAME and injected into the GC to check for the presence of any trans fats. Nine different concentrations from 0.2% to 5% were prepared to obtain the calibration curve, which was established between the integrated area and trans concentration. High R^2 value of more than 0.99 was obtained. Fresh oils were used as the reference background for oil samples, whose spectra were collected and saved. A drop of neat oil sample without weighing was placed on the crystal and the obtained spectrum of the test sample was ratioed against the spectrum of the corresponding reference material. The area between 990 and 945 cm^{-1} was integrated using software Spectrum Version 5.0.1 and compared against the calibration standards using linear regression to determine the amount of trans fats.

2.9. Attenuated total reflection infrared spectroscopy – negative second derivative

Details of the method were described in Milosevic et al. (2004). Briefly, a set of calibration standards was prepared in the range of 0.2–5%. The spectra of the test samples were measured against air. Height values of the negative second derivative of the absorption spectra were used for quantification. A R^2 value of more than 0.99 was obtained for the calibration curve.

2.10. Extraction of fat from frozen French fries

Fat from the pre-frozen French fries was extracted using a Soxtec 2050 auto extraction unit (Foss Tecator AB, Höganäs, Sweden). One gram of homogenized French fries sample was weighed and then dried in an oven at 115 °C for 2 h. Petroleum ether (B.P. 35–60 °C) was selected as the extraction solvent. The following operating conditions were used: temperature at 135 °C; boiling time of 20 min; rinsing time of 40 min; and recovery time of 10 min. Solvent from the extracted fat was evaporated under vacuum. The resultant fat was converted to its FAME and then injected to GC for further analysis.

2.11. Statistical analysis

All experiments were performed in triplicates. Two-factor ANOVA was applied to test statistically significant difference or similarity between the mean values of trans fats obtained by ATR AOCS method, −2D ATR method and GC-FID method at 5% significance level. Correlations between the above stated three methods were also calculated. Linear regression was used to establish equations for calibration curves in ATR AOCS method and −2D ATR method. Microsoft Excel 2003 was used to perform the above stated statistical analyses.

3. Results and discussion

3.1. Optimization of GC operating conditions

A number of operating conditions were optimised to achieve the best separation. The isothermal temperature condition at the oven temperature of 180 °C with the carrier gas flow rate at 1 ml/min, as per specified by AOCS Official Method Ce 1 h-05, was also tested but the achieved separation was poor. A better separation was achieved in trans 18:1, trans 18:2 and trans 18:3 regions under the temperature program conditions as stated earlier than using an isothermal run. A sample chromatogram of linoleic

standards is presented in Fig. 1 following the same operating parameters. A clean separation of all the four trans 18:2 isomers was observed. Similar chromatograms with high resolution for all the other standards were also obtained. As the capillary column used was cyanopolysiloxane stationary phase, trans fats eluted earlier than their corresponding cis isomers, e.g. 9-trans 18:1 eluted before 9-cis 18:1. 9-Cis, 12-trans and 9-trans, 12-cis eluted before 9-cis, 12-cis 18:2 isomers. The major trans fatty acids found in the samples were 9-trans 18:1, 9-cis, 12-trans and 9-trans, 12-cis 18:2 and cis-9, cis-12, trans-15 and 9-trans, 12-cis, 15-cis 18:3 isomers. The isomers were summed up as 18:1, 18:2 and 18:3 trans after quantification.

3.2. Validation of FAMEs

As free fatty acids were not present in substantial amounts in all the samples, potassium methoxide methylation procedure was selected for the preparation of FAMEs. To evaluate the methylation procedure, recovery rate of triolein was compared with that of standard methyl oleate. The recovery rate was found to be 97% with CV of 1.6%, thus validating the effectiveness of the selected methylation procedure.

3.3. Fatty acid composition of fresh oils and prefried French fries

The major fatty acids in unused (i.e. fresh) PO and CO were oleic acid, palmitic acid and linoleic acids, whereas linoleic acid and oleic acid were the major components in fresh SO. The detailed fatty acid compositions of all the three oils in their unused forms are presented in Table 1.

Fat extracted from the pre-fried and frozen French fries contained 11.28 ± 0.67 mg/g of palmitic acid, 11.18 ± 0.94 mg/g of stearic acid, 23.44 ± 1.50 mg/g of 18:1 trans isomers, 28.00 ± 1.62 mg/g of oleic acid and 6.20 ± 0.50 mg/g of linoleic acid. Trans isomers of linoleic and linolenic acids were not found in the fat. The above composition was very similar to the fatty acid profile of partially hydrogenated soybean oil as reported in Karabulut, Kayahan, and Yaprak (2003). This indicates that the fat used for pre-frying French fries was possibly the same, i.e. partially hydrogenated soybean oil.

3.4. ATR calibration

Nine different concentrations of trielaidate in triolein were prepared. The following linear regression equations were found for the two ATR methods:

3.4.1. AOCS method Cd 14d-99

$$y = 0.01623x + 0.00127 \quad (R^2 = 0.9981) \tag{1}$$

where y is the area integrated between 990–945 cm^{-1} and x is the percent trans (wt. basis) of trielaidate in triolein.

3.4.2. −2D method

$$y = 0.000043x + 0.000030 \quad (R^2 = 0.9953) \tag{2}$$

where y is the absorbance read from the negative second derivative absorbance spectra and x is the percent trans (wt. basis) of trielaidate in triolein.

The use of a grid system to directly read out the approximate value of trans fats in a sample as compared to that in the standards was recommended in the originally proposed method (Milosevic et al., 2004). However, as this method only provides the range of the trans fats, but not the absolute value, a calibration curve using linear regression was prepared in our study.

One of the findings reported in the studies on the −2D method was the presence of around 0.5% (determined by GC) trielaidate contaminant in commercially available triolein. Therefore, commercially available trioelin was not recommended for preparing calibration standard mixtures (Milosevic et al., 2004). Thus in our study, a purity check of triolein was done using GC-FID analysis. It was found to be free of trans fatty acids.

3.5. Recovery rates by ATR spectroscopy

The ability of both ATR techniques to quantify trans fats was measured by adding known amounts of trielaidate to all the three fresh oils. Quantification of trielaidate in the resultant mixture was then performed utilizing both area and −2D methods.

It was mentioned earlier that in order to obtain the best ratioing in the ATR AOCS area method, the reference background should match the fatty acid composition of the samples. Therefore the reference backgrounds selected for the samples were their corresponding oils in fresh form despite of the minute amount of trans fats in the fresh oils as shown in Table 1.

For the −2D ATR method, trans fats present in the corresponding fresh oil samples were subtracted from the amount of trans fats in the spiked samples obtained by −2D absorption spectra, as the reference background used during the measurement was air and those initial trans fat amounts were not taken into account in the measurement of the spiked samples.

The samples were spiked in the range of 6.05–13.98 mg/100 mg of oil samples. The recovery rates were found to be in the range of

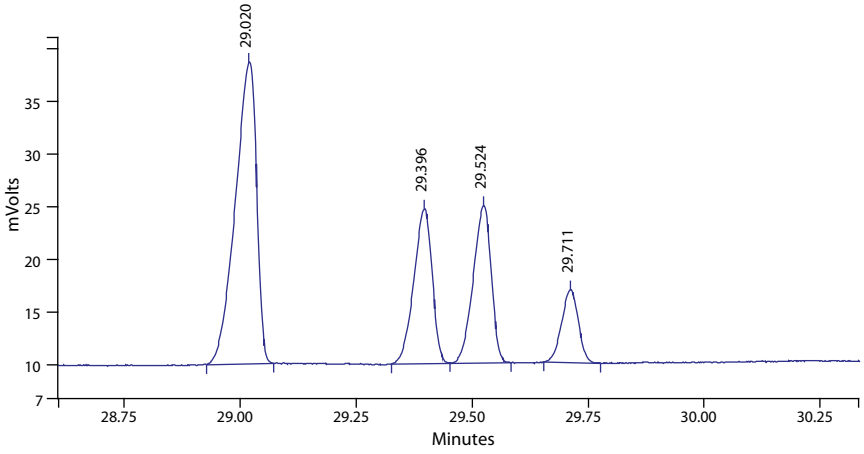

Fig. 1. Gas chromatogram of a standard FAME mixture of trans-9, trans-12; cis-9, trans-12; trans-9, cis-12 and cis-9, cis-12 octadecadienoic acid methyl ester.

84–115% (Table 2). Recovery rates in the range of 89.3–100.3% have been reported in another study, in which mixtures were prepared by adding known amounts of methyl elaidate to canola oil FAMEs and the spactra of the mixtures were ratioed against the spectrum of canola oil FAMEs (Ali et al., 1996).

3.6. Trans fats in frying oil samples

The amounts of trans fats determined by the GC-FID method were considered as the true values. The major trans fatty acid isomers found in PO and CO before the start of frying were 9-cis, 12-trans C18:2, 9-trans, 12-cis C18:2, cis-9, cis-12, trans-15 C18:3 and 9-trans, 12-cis, 15-cis 18:3. However, trans isomers of 18:3 were not found in SO. Elaidic acid was not found in fresh oil samples. Similar observations on trans fatty acids in refined oils have been reported in Greyt, Kint, Kellens, and Huyghebaert (1998). The abundance of trans linoleic acids in sunflower oil has also been reported by Romero et al. (2000). Sebedio and Ratnayake (2008) reported that the two mono-trans isomers of linoleic acid (i.e. 9-cis, 12-trans C18:2 and 9-trans, 12-cis C18:2) were present at similar levels and very often higher than the trans isomer 9-trans, 12-trans C18:2 in many non-hydrogenated dietary fats.

The initial amounts of total trans fatty acids in our samples were in the range of 5.40–8.84 mg/g (Table 3). Greyt et al. (1998) reported that the range of total trans fatty acids in refined oils (soybean oil, corn oil, sunflower oil, high oleic sunflower oil, low erucic rapeseed oil and high erucic rapeseed oil) was between 0.15% and 6.03%.

Linear relationships between the amount of elaidic acid and the number of frying cycles were established in all the three frying batches of PO-FF, CO-FF and SO-FF (Fig. 2a). At the end of 40 frying cycles, the amount of elaidic acid was found to be the highest in CO-FF, followed by SO-FF and PO-FF. The highest slope was also observed in the case of CO-FF (Fig. 2a).

For the trans isomers of linoleic and linolenic acids, no statistically significant difference among various numbers of frying cycles

Table 2
Percentage trans, determined as trielaidin per total triacylglycerol (TAG) for spiked oil samples with varying amounts of trielaidate.

	Trielaidate amount added (g)	Oil sample wt (g)	Calculated trans (mg/100 mg)	Trans by ATR AOCS method[a] (mg/100 mg)	Recovery (%)	Trans by −2D ATR method[a,b] (mg/100 mg)	Recovery (%)
SO	0.009	0.095	9.37	9.59 ± 0.051	102	8.25 ± 0.433	88.1
	0.011	0.076	14.0	13.0 ± 0.093	93.1	15.5 ± 0.750	111
PO	0.006	0.101	6.05	5.19 ± .027	85.8	6.08 ± 0.330	101
	0.010	0.109	9.50	7.99 ± 0.142	84.1	10.0 ± 0.250	105
CO	0.007	0.100	7.30	7.70 ± 0.374	105	7.17 ± 0.144	98.2
	0.011	0.104	10.5	12.1 ± 0.310	115	10.8 ± 0.669	103

[a] Average values of three readings on the same spiked sample.
[b] Trans fatty acids present in fresh oil samples were subtracted from the trans fats obtained by −2D ATR method as the reference background used was air.

Table 3
Amount of total trans fats obtained in frying oil and control oil samples using different approaches.

No. of frying cycles	Total trans fat (mg/g) in frying oil samples			Total trans fat (mg/g) in control samples		
	ATR (AOCS method)	ATR (−2D method)	GC-FID method	ATR (AOCS method)	ATR (−2D method)	GC-FID method
		PO-FF			PO	
0	0.00 ± 0.00	4.91 ± 0.46	5.80 ± 0.54	0.00 ± 0.00	3.00 ± 0.03	6.26 ± 0.32
5	0.25 ± 0.02	5.41 ± 0.42	7.44 ± 0.53	1.21 ± 0.003	3.75 ± 0.04	6.97 ± 0.23
10	2.70 ± 0.31	7.83 ± 0.57	6.65 ± 0.54	1.74 ± 0.05	5.00 ± 0.04	5.29 ± 0.20
15	3.71 ± 0.46	9.08 ± 0.52	9.83 ± 0.74	2.54 ± 0.01	6.25 ± 0.08	10.2 ± 0.53
20	5.03 ± 0.40	10.1 ± 0.87	10.2 ± 0.42	2.87 ± 0.05	7.75 ± 0.06	9.57 ± 0.35
25	6.80 ± 0.20	11.3 ± 0.75	10.2 ± 0.32	4.43 ± 0.06	8.00 ± 0.10	7.98 ± 0.30
30	7.24 ± 0.85	13.6 ± 0.52	12.1 ± 0.76	5.33 ± 0.05	7.00 ± 0.09	8.21 ± 0.45
35	8.92 ± 0.32	15.8 ± 1.12	11.4 ± 0.89	6.82 ± 0.10	10.5 ± 0.09	8.23 ± 0.27
40	13.0 ± 0.09	17.6 ± 0.72	12.1 ± 0.45	7.40 ± 0.08	11.3 ± 0.1	8.79 ± 0.20
		CO-FF			CO	
0	0.00 ± 0.00	5.33 ± 0.80	5.47 ± 0.54	0.00 ± 0.00	5.33 ± 0.80	5.40 ± 0.40
5	2.40 ± 0.06	5.00 ± 0.21	4.92 ± 0.39	2.35 ± 0.31	6.50 ± 0.32	6.31 ± 0.31
10	5.25 ± 0.27	8.50 ± 0.43	7.41 ± 0.70	3.17 ± 0.14	6.25 ± 0.20	6.29 ± 0.21
15	7.82 ± 0.31	11.9 ± 0.52	8.98 ± 0.31	4.59 ± 0.23	4.25 ± 0.15	6.48 ± 0.30
20	11.4 ± 0.15	15.5 ± 1.39	15.4 ± 0.64	4.49 ± 0.16	6.50 ± 0.23	6.23 ± 0.25
25	14.7 ± 1.15	16.0 ± 0.75	13.8 ± 0.26	7.66 ± 0.21	7.75 ± 0.17	5.51 ± 0.40
30	16.6 ± 0.53	20.9 ± 0.38	15.7 ± 0.61	7.69 ± 0.31	8.75 ± 0.27	8.06 ± 0.21
35	21.8 ± 0.68	23.8 ± 1.00	17.6 ± 0.83	7.64 ± 0.54	12.3 ± 0.56	9.16 ± 0.10
40	25.2 ± 0.51	28.3 ± 1.77	19.4 ± 1.15	9.51 ± 0.75	12.8 ± 0.87	9.46 ± 0.15
		SO-FF			SO	
0	0.00 ± 0.00	10.2 ± 0.14	8.84 ± 0.43	0.00 ± 0.00	11.8 ± 0.10	8.40 ± 0.53
5	5.68 ± 0.26	8.08 ± 0.28	11.8 ± 0.51	3.14 ± 0.15	8.50 ± 0.27	5.73 ± 0.47
10	7.75 ± 0.27	10.5 ± 0.43	7.84 ± 0.34	3.08 ± 0.17	9.00 ± 0.26	7.62 ± 0.32
15	13.9 ± 0.44	13.3 ± 0.00	13.0 ± 0.55	5.65 ± 0.14	10.5 ± 0.00	7.10 ± 0.30
20	15.5 ± 0.30	14.9 ± 1.00	15.2 ± 0.65	6.77 ± 0.30	12.8 ± 0.53	6.26 ± 0.41
25	13.3 ± 0.32	15.0 ± 0.43	14.9 ± 0.86	8.87 ± 0.18	14.5 ± 0.15	6.72 ± 0.45
30	16.8 ± 0.84	22.8 ± 0.31	19.2 ± 1.20	10.4 ± 0.36	15.0 ± 0.41	11.8 ± 0.56
35	20.2 ± 0.28	25.2 ± 0.14	20.1 ± 1.00	12.1 ± 0.40	17.0 ± 0.11	12.5 ± 0.70
40	19.3 ± 0.40	27.8 ± 0.00	20.1 ± 1.19	13.4 ± 0.41	20.0 ± 0.14	13.4 ± 0.85

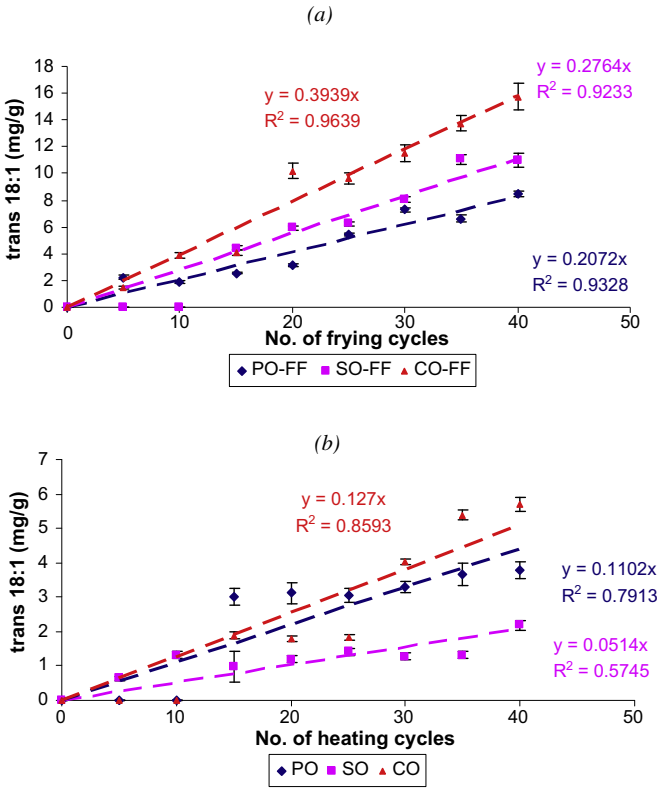

Fig. 2. Amount of trans 18:1 (mg/g) fatty acids vs. number of frying cycles in (a) oil samples with French fries as fried food and (b) under controlled heating conditions.

was found in all the batches ($p = 0.05$). The final amounts of total trans fats after 40 frying cycles in PO-FF, CO-FF and SO-FF were 12.08 ± 0.45, 19.38 ± 1.15 and 20.08 ± 1.19 mg/g of oil, respectively.

Aladedunye and Przybylski (2008) observed changes in trans fatty acids during the frying of pre-fried French fries in canola oil at two different temperatures including 185 ± 5 °C and 215 ± 5 °C. They found that after 7 days of frying (with 7 h frying per day), the trans fatty acid content increased from its initial value of 2.4–3.3% and 5.9% at 185 ± 5 °C and 215 ± 5 °C, respectively. Another study on the production of trans fatty acids in extra virgin olive oil, high oleic sunflower oil and the regular sunflower oil suggested that the amount of trans fats produced was less than 5 mg/g in all the oils after 20 frying cycles with various frozen foods (Romero et al., 2000).

3.7. Trans fats in control oil samples

In the heated oil samples, the final concentration of total trans fatty acids in PO, CO and SO were 8.79 ± 0.20, 9.46 ± 0.15 and 13.40 ± 0.85 mg/g, respectively (Tables 3). Elaidic acid appeared after 15 heating cycles in PO and CO, whereas in SO it could be found after 5 cycles of heating. Once produced, the amount of elaidic acid was found to be very stable up to 25 frying cycles in PO and CO (Fig. 2b). After 30 cycles of heating, significant increases in elaidic acid were observed again in both PO and CO, however in the case of SO, elaidic acid produced after 10 frying cycles was quite stable afterwards throughout the repeated heating process.

Higher amounts of elaidic acid in frying oil samples as compared to control oil samples could be explained by the release of these trans fatty acids from the pre-fried frozen foods into the oil. Since the French fries used in our experiments were high in 18:1 trans content, frying oil samples had more 18:1 trans content compared to heated samples. It was reported that about 80% of the fat from pre-fried frozen potatoes could be released to frying oil in batch fryers (Pozo-Diez, Masoud-Musa, Perez-Camino, & Dobarganes, 1995).

A substantial increase was observed in the trans isomers of linoleic acid in SO samples. 9-cis, 12-trans C18:2 isomer increased from 4.48 ± 0.25 mg/g in the unused oil to 6.23 ± 0.21 mg/g after 40 cycles of heating, whereas 9-trans, 12-cis C18:2 isomer increased from 0.61 ± 0.05 mg/g in fresh oil to 5.09 ± 0.31 mg/g. No statistically significant difference was observed for these linoleic acid trans isomers in PO and CO batches ($p = 0.05$). It has been reported earlier that simple heating in a deep frying process could result in the isomerisation of linoleic and linolenic acid double bonds from cis to trans form (Grandgirard et al., 1984). Moreno et al. (1999) reported that intensive heating of oils caused an increase in trans fatty acids; and the amount of trans fatty acids increased from 0.23% to 1.10% when sunflower oil was heated at 200 °C for 40 min whereas it increased to 3.20% when heated at 250 °C for the same duration.

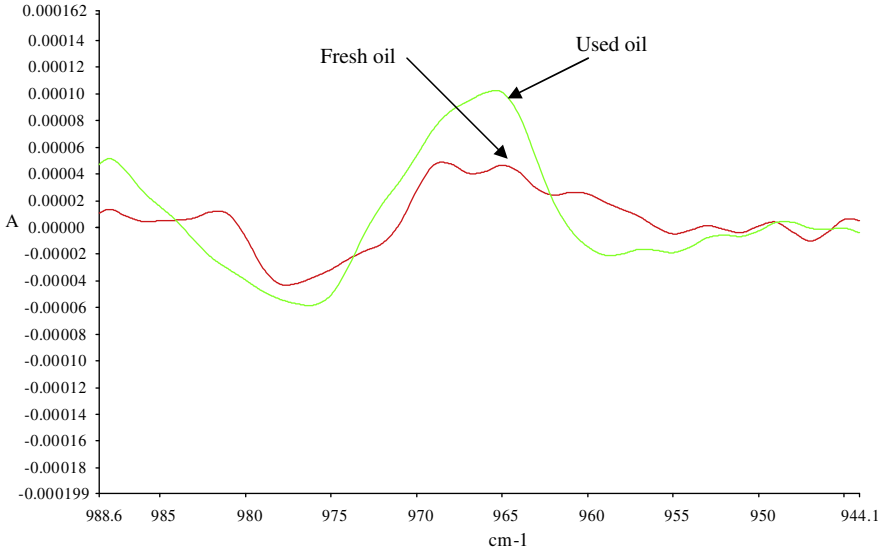

Fig. 3. Trans band region in negative second derivative spectra of fresh palm olein and used palm olein (after 40 frying cycles).

3.8. Comparison among different methods for evaluating trans fats

Generally, trans contents determined by the ATR AOCS method were lower as compared to the actual amounts analysed by the GC-FID method, whereas the amounts of trans fatty acids found by the −2D ATR method were higher in comparison with the values obtained by the GC-FID method. As the reference background used in the ATR AOCS method was the corresponding oil samples, the method did not take into account the amount of trans fatty acids present in the fresh oil, thus producing lower amounts when compared with the GC-FID method. On the other hand, the −2D ATR method included the trans fatty acids present in the original fresh oil samples, as the reference used was air instead of fresh oils (Fig. 3). As suggested by the researchers who proposed this −2D ATR method (Milosevic et al., 2004), the slowly undulating baseline under the trans fat peak was found to be minimal, since the second derivative favoured higher frequency components in Fourier decomposition of the spectrum. The higher values exhibited by the −2D ATR method might be due to the presence of a residual triacylglycerol absorption band in the 966 cm^{-1} region.

ANOVA analysis showed that the mean trans values obtained by the ATR AOCS and −2D ATR methods were statistically different at 5% significance level in all the six batches of experiments. However, high correlation was found between the two methods for all the cases ($r = 0.80–0.99$). Milosevic et al. (2004) reported high correlations between the ATR AOCS method and the −2D ATR method ($r = 0.85$) for a variety of samples containing trans fats in the range of 0.00–6.43%.

The mean trans values obtained by the ATR AOCS method were statistically different when compared with the mean trans values obtained by the GC-FID method in five of the six batches of experiment ($p = 0.05$). Only for the control samples of PO, statistically same mean trans values were yielded by the ATR AOCS and GC-FID methods ($p = 0.05$). Poor ($r = 0.37$) to high correlations ($r = 0.96$) were found between the results obtained by the ATR AOCS and GC-FID methods. The lowest correlation of 0.37 was found for the control PO samples, whereas for all other samples high correlations in the range of 0.75–0.96 were yielded. These results are in contrast to those of Adam, Mossoba, Dawson, Chew, and Wasserman (1999), which showed that the GC and ATR AOCS method determinations were in good agreement and the accuracy was generally high by both the methods. The ratio of mean trans values determined by the two methods, ATR/GC, was at 0.85, 1.04, and 1.01 for test samples having trans levels of about 0.7%, 8%, and 38%, respectively.

The mean trans values obtained by the −2D ATR and GC-FID methods were found to be statistically the same in four of the six batches, whereas statistically different results were found for COFF and control SO batches at 5% significance level. High correlations ($r = 0.83–0.97$) were found between the trans values by the −2D ATR and GC-FID methods in five of the six batches. Only for the control batch of PO a low correlation of 0.56 was found between the results obtained by the −2D ATR method and those by the GC-FID method.

4. Conclusions

Both heating and frying processes induced production of elaidic acid, which was absent in the fresh oil samples. In comparison to heating, frying produced more of 18:1 trans fatty acids. Higher amounts of elaidic acid in the frying oil samples were attributed to the release of trans fats from the pre-fried frozen French fries to the oil. Among the three different methods used to quantify trans fats, while the GC-FID method provided detailed information on fatty acid composition, the two ATR methods provided quantification of total trans fats rapidly. The ATR AOCS method produced lower trans values and the −2D ATR method produced higher trans values when compared with those obtained by the GC-FID analysis. The −2D ATR method was found to provide statistically similar results as those by the GC-FID method in more batches as compared to the ATR AOCS method.

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