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Trans Fatty Acid Analysis of Frying Oil Using ATR-FTIR Spectroscopy: A Study on Indian Traditional Snack Foods

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HIGHLIGHTS

- Trans Fatty Acid (TFA) content in oils fried at 4 h was significantly higher than the ones at 0 and 2 intervals.
- Four percent of Samosa fried oils and 17.4% of Jalebi fried oils were over the maximum allowed limit of TFA.
- The increase in frying time decreased the peroxide values and increased saturated fatty acids values of oils.
- Vendors and consumers should be educated by regulatory authorities regarding health risk of TFA in street fried snacks.

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Acronyms and abbreviations
ATR-FTIR=Attenuated Total Reflection-Fourier Transform Infrared
TFA=Trans Fatty Acid

ABSTRACT

Background: Trans Fatty Acid (TFA) content in oil is an important quality parameter due to its adverse health effect. This study was aimed to examine the TFA content in the frying oil used by street food vendors in India for two traditional snack foods.

Methods: Totally, 143 oil samples were collected at different frying times (0, 2, and 4 h) from five different vendors for Samosa and Jalebi. TFA levels of the oil samples were analyzed by Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR). Statistical analyses were carried out using SPSS software version 23.0.

Results: ATR-FTIR spectra exhibited an increase in peak intensity at 966 cm^{-1} with different frying time in both frying oil samples, indicating the formation of TFA. The TFA content in oils fried at 4 h was significantly higher than the ones at 0 and 2 intervals. It was found that 3 out of 74 (4%) Samosa fried oils and 12 out of 69 (17.4%) Jalebi fried oils were over the maximum allowed regulatory limit of TFA (5%). Jalebi fried oils had significantly higher TFA content than Samosa fried oils.

Conclusion: The increase in frying time decreased the peroxide values and increased saturated fatty acids and TFA values of oils used for both food items. The local vendors and consumers should be educated by national authorities regarding health risk of TFA in street fried snacks.

Introduction

Fried foods have unique sensory properties which make them highly palatable for the consumers. The fried food quality depends on both frying condition and the heating medium (Weisshaar, 2014) in which they are fried, i.e. oils and fats. In fried food preparation, simultaneous mass and heat transfer as well as texture and flavour characteristics of frying medium depends on the quality of frying oil (Debnath et al., 2012).

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During deep fat frying, food material is immersed into the heated oil approximately 170-200 °C. On repeated frying at high temperature, oil go through a sequence of intricate chemical reactions, including lipid hydrolysis, oxidation, polymerization, and isomerization. These reactions produce few harmful compounds like heterocyclic amines, trans fat, and polar compounds that degrade the frying oil quality (Sayyad, 2017; Wang et al., 2015). During the frying process, there is an increase in polar compounds, free fatty acids, and Trans Fatty Acids (TFAs) whereas unsaturated fatty acids decrease in the oil (Choe and Min, 2007).

TFA decreases the amount of High Density Lipoprotein (HDL) and increases the Low Density Lipoprotein (LDL). Both LDL and HDL are the main risk factor for the coronary heart disease (McCarthy et al., 2008). Furthermore, TFA consumption increases the risk of type 2 diabetes (Bhardwaj et al., 2011; Mozaffarian et al., 2006). World Health Organization (WHO) recommends that intake of TFA contents should be limited to less than 1% of the total energy intake (Uauy et al., 2009). Some developed countries mandate the labeling of TFA content on food product. In India, Food Safety and Standards Authority of India (FSSAI) has set the limit of the trans fat content to 5% in inter-esterified vegetable fat/oil, margarine, fat spreads, and also hydrogenated vegetable oils (Maindola, 2019).

Samosa and Jalebi are the fried snacks which are very popular street foods in North India. Samosa is a refined wheat flour product with potato stuffing (Raj et al., 2017) and Jalebi is a product made with fermented batter of refined wheat flour fried in oil having sweet, crispy, glossy, and juicy taste (Balaswamy et al., 2012). These food items are generally prepared and sold at public places for immediate consumption (Imathiu, 2017). Street food is cheap, convenient, and often nutritious food for low income group. Mostly, street food vendor use the frying oil in a repetitive manner for frying due to lack of proper food safety knowledge and to cut down the cost. There is no specific criterion to determine the number of times specific oil can be used for frying without compromising its quality. A recent Indian study on the quality assessment of oil used by 44 street vendors in two low-income villages in Haryana and an urban slum in Delhi reported that the frying oil was containing 25-69% saturated fats and 0.1-30% of TFAs (Gupta et al., 2016). According to national regulation in India, oils should not be used after 2-3 times frying. However, street food vendors and restaurants often reuse oil for frying.

With the recent studies on the fried foods and oils, safety and quality of oils used by street food vendors have become the important issues. Till date, several studies have been conducted to quantify TFA of fried foods produced in different countries (Karunathilaka et al., 2018; McCarthy et al., 2008; Mossoba et al., 2015; Zahir et al., 2017); however, to the best of our knowledge, no reports are available on easy and quick method to quantify TFA of these selected street food frying oils. Therefore, the main objective of this study was to quantify TFA of Indian street food frying oils. Based on food regulatory authorities of many countries (Firestone, 2007), TFA analysis of oil used for frying the street snack items was considered as quality parameter in this study. Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy was used for rapid quantification of TFA in the oil samples.

Materials and methods

Chemicals

All chemicals used were analytical grade. Elaidic acid (≥95% purity) was purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan). Triolein (≥99% purity) and iso propanol-2 (≥99% purity) were purchased from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India).

Sample collection

About 12-15 ml oil samples were collected during 5 days in March 2018 from 5 different Samosa and Jalebi street vendors in Sonipat, Haryana, India. Daily, 3 samples were collected at a constant time interval of 2 h (0, 2, and 4 h) during the frying process of 4 h. All samples were collected in triplicates. A total of 143 frying oil samples were collected which were used for preparation of Samosa (n=74) and Jalebi (n=69). The original type of these oils was palm oil (n=120) and vanaspati oil (n=23). The samples were stored in a refrigerator (4 °C) before further analysis. The temperature of the oil at the time of sample collection ranged from room temperature to 300 °C.

Physical property of fried oil

Frying oil quality would depend on the physical parameters involved, including type of oils, frying type, amount of product fried at a given time, frying pan material, oil reusability frequency, etc. Therefore, it is essential to understand these physical parameters followed by street vendors for preparing the selected food items. During oil sample collection from the street vendors, data for physical parameters were collected with a simple questionnaire though interview mode. These questions were included: i) what was the type of oils used? ii) what type of frying condition was used? iii) how much the total amount of food was fried per day by the vendors? iv) what type of material was made in frying pan ? v) whether oil was

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discarded every day? and vi) whether oil was replenished on the same day?

ATR -FTIR analysis

The absorption spectra of all collected samples were analyzed by using FTIR spectrometer with Diamond crystal cell ATR (path length: 1.66 µm; Bruker Optics, Model-Alpha, Germany) at 4 cm^{-1} resolutions, 0.2 cms^{-1} scan speed, and 24 scans of each sample at 30 °C in the wavenumber range of 4000-600 cm^{-1} (Jha et al., 2015). After each sample scanning process, the ATR crystal was cleaned using a soft tissue paper with iso-propanol-2. Background spectra were collected after each sample scanning and automatically deducted from sample spectra. An OPUS spectroscopy software version 7.2 was used for FTIR data collection and processing. To obtain ideal calibration models and to remove the spectral interference various pretreatment have been performed on spectral data. The pretreatment that have been used were Baseline correction, 1^{st} derivative, 2^{nd} derivative, and Standard Normal Variate (SNV).

The change in intensity and position in the characteristic peaks of different regions of FTIR spectrum was used to determine the quality of frying oil like peroxide value, degradation of oil through hydrolysis, etc. So, FTIR spectra of frying oil at different time interval of frying were also recorded for quality analysis of frying oil.

Quantification of TFA

The TFA in the oil samples were evaluated by second derivative ATR-FTIR spectra (Galvín et al., 2016; Mossoba et al., 2015). A set of 5 calibration standards were prepared by mixing elaidic acid into trans free reference fat (triolein) (0, 2.5, 05, 7.5, and 10%). The standards were melted before the preparation of the calibration curve. Each sample was analyzed in triplicates. A calibration curve of absorbance height at 966 cm^{-1} versus elaidic acid, TFA standard concentration was generated and a linear regression equation was obtained. The TFA value (%) of test samples were quantified by substituting the absorbance height of second-derivative trans band into the equation (Mossoba et al., 2011).

Statistical analysis

In order to understand statistical difference between the TFA content of Samosa and Jalebi fried oil at different time intervals, z-test and t-test was performed. TFA content in Jalebi and Samosa fried oils were compared through statistical analysis of z-test. Further, difference in TFA between Samosa and Jalebi fried oil at different time intervals (0, 2, and 4 h) was also statistically analysed using t-test. All the statistical analyses were carried out using SPSS software version 23.0. Significance level was considered as $p<0.05$.

Results

It was observed that most of the street food vendors used palmolien oil for frying due to its low cost, while very few vendors used other vegetable oils like soybean oil. It was observed that intermittent frying is the common frying method adopted by all the street food vendors. Most of the vendors used the iron pan for frying which may accelerate the oxidation of frying oil. It was also found that frying temperature remained above 220±10 °C. Most of the vendors did not use complete fresh oil during frying rather they reused it with the further addition of fresh oil (Table 1).

FTIR spectra, in the range of 4000-600 cm^{-1}, pertaining to Samosa and Jalebi frying oil samples collected at different frying time has been given in Figure 1. Irrespective of intermittent frying duration, no shift was observed in the position of the bands. However, the changes in the peak absorbance values were significant ($p<0.05$). It was observed that there was a net decrease in the peak absorbance value at 3006, 2922, 2853, 1745, 1654, and 721 cm^{-1} with the frying time while increase in the peak absorbance value at 966 and 3471 cm^{-1}.

The major changes were observed in the regions 3050-2800, 1745-1654, and 1000-900 cm^{-1}. It was observed that as the frying time increased, there was a decrease in the peak intensity at 3006, 2922, and 2853 cm^{-1}. FTIR spectra exhibited a decrease in the absorbance peak at 1745 and 1654 cm^{-1} with respect to different frying time (0, 2, and 4 h) in the collected oil samples from both the Samosa and Jalebi street vendors. At 966 cm^{-1}, an increase in peak intensity was noted with respect to different frying time. Furthermore, a decreasing pattern of peak was observed at 1163 cm^{-1} for the samples collected at 2 and 4 h frying time interval from both the street vendors. Peak absorbance at 721 cm^{-1} decreased with the frying time (Figure 1).

The TFA of collected oil samples were quantified through the ATR-FTIR spectroscopy. FTIR spectra of different concentration of TFA standards are illustrated in Figure 2. TFA content was found to increase with frying time in both Samosa and Jalebi fried oil samples (Figure 3). The TFA content ranges during 0, 2, and 4 h of frying in Samosa fried oil samples were 1.56-3.25, 1.94-3.56, and 2.29-3.82%, respectively; while these rates in Jalebi fried oil were 1.43-3.70, 2.49-4.91, and 3.98-5.40%, respectively. From our TFA analysis data, it is clear that irrespective of the food samples used for frying, the formation of TFA was increased with increasing duration of frying.

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According to Indian food regulations, TFA must be below 5% in the fried oils. The oil samples for both food items collected after 2 h of frying interval were found to have TFA under the regulatory limit; however, some samples collected at 4 h of frying exceeded the regulatory limit. Statistical analysis showed that the TFA content in oils fried at 4 h was significantly (p=0.0000) higher than the 0 and 2 intervals. It was found that 3 out of 74 (4%) Samosa fried oils and 12 out of 69 (17.4%) Jalebi fried oils were over the maximum allowed regulatory limit of TFA. Based on statistical analysis, Jalebi fried oils had significantly (p=0.0015) higher TFA content than Samosa fried oils.

Discussion

In the present research, we showed considerable TFA content in fried oils used in preparation of two common Indian snack foods. Similarly, Debnath et al. (2012) reported the changes in fatty acids composition, total polar material, and peroxide value of oils during intermittent frying. Also, Das et al. (2013) found that compared to continuous frying, intermittent frying caused more degradation of oil. The reason for this degradation in case of intermittent frying was attributed to the higher solubility of oxygen in oil when it cools below its frying temperature. While in the continuous frying, steam covers the surface of the oil and prevents the oil contact with the atmospheric oxygen. It results in less degradation in oil quality. Therefore, there might be high degree of degradation of oils used by the Indian street food vendors, as we found that all of them used intermittent frying process (Table 1). Apart from the intermittent frying process, previous studies stated that frying oil quality may be also affected by many factors like number of batches fried, oil replenishment, fried food composition, frying temperature and time, etc. (Choe and Min, 2007; Mariod et al., 2006; Matthäus, 2006; Normand et al., 2006).

A decrease in peak intensity at 3006 cm^{-1} could be assigned to C-H stretching vibration of the cis double bond (=C-H). This shows a net loss of cis double bond with an increase in frying time (Siddique et al., 2015). In other words, there might be an increase in the saturation of oil with an increase in the frying time. Recent studies revealed the same spectral trend at 3006 cm^{-1} during heating of soybean oil at different time interval (Poiana et al., 2015). It is also reported that the information about the saturation and unsaturation level in vegetable oil can be obtained from the peak absorbance at 3006 cm^{-1} (Marina et al., 2013; Poiana et al., 2015).

Table 1: Profile of the frying process used by the street food vendors in Sonipat, Haryana, India

Variables		Absolute frequency (N)		Relative frequency (%)	
		Samosa	Jalebi	Samosa	Jalebi
Type of the oil used					
Soybean oil		1	0	20	0
Vanaspati (hydrogenated fat)		0	2	0	40
Palmolein oil		4	3	80	60
Any other oil		0	0	0	0
Types of frying					
Continuous		0	0	0	0
Intermittent		5	5	100	100
Amount of food fried Per day in 4 h frying time					
Samosa (pcs.)	Jalebi (kg)				
250	10	1	1	20	20
250-500	10-15	3	2	60	40
500-750	15-20	1	2	20	40
>800	>20	0	0	0	0
Frying pan base material					
Iron		5	5	100	100
Copper		0	0	0	0
Stainless steel		0	0	0	0
Oil replenishment at the same day					
Yes		0	4	0	80
No		5	1	100	20
Oil discarding					
Yes		0	0	0	0
No		5	5	100	100

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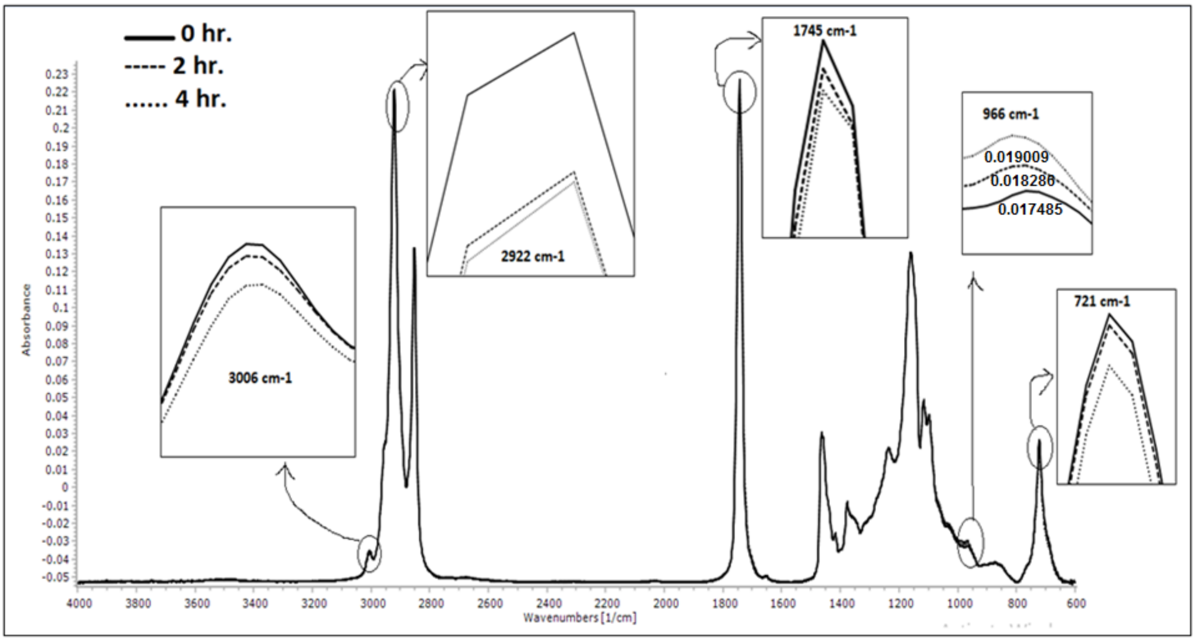
(A)



(B)

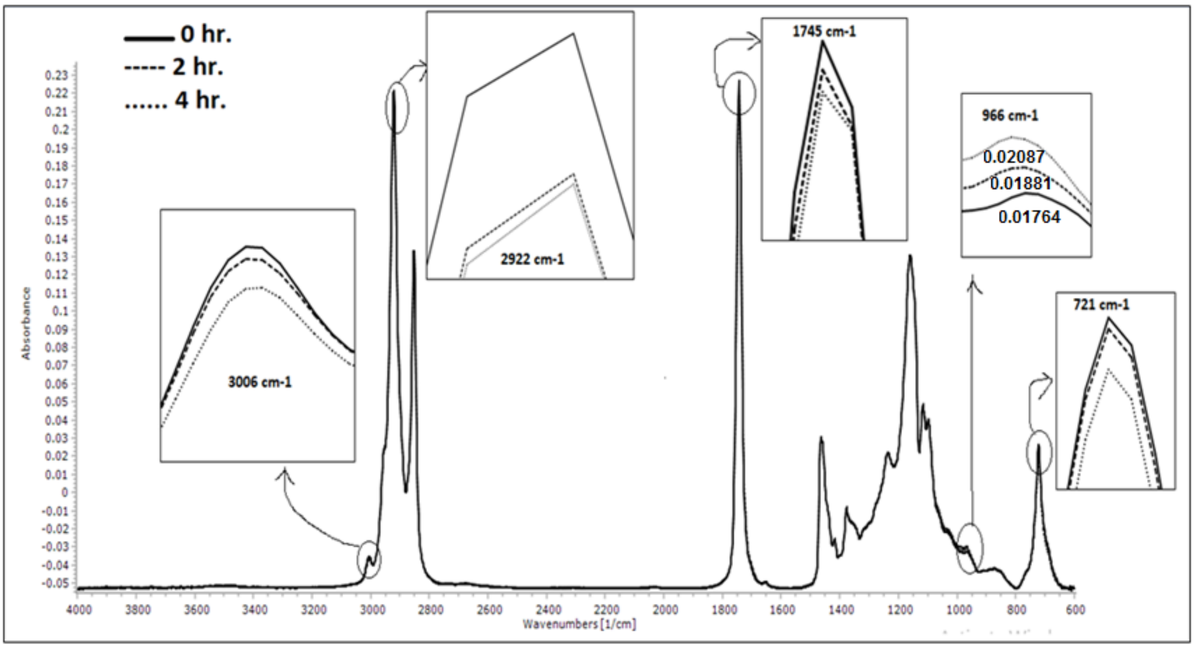


Figure 1: Stacked FTIR spectra of fried oil samples pertaining to Samosa (A) and Jalebi (B) at different frying time (0, 2, and 4 h)

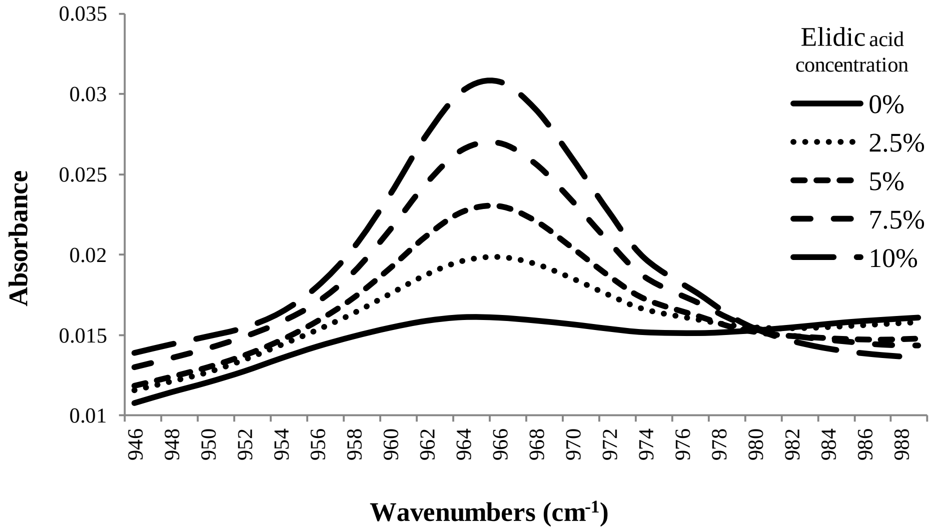


Figure 2: Spectra of reference standard from 0 to 10% trans fatty acid content

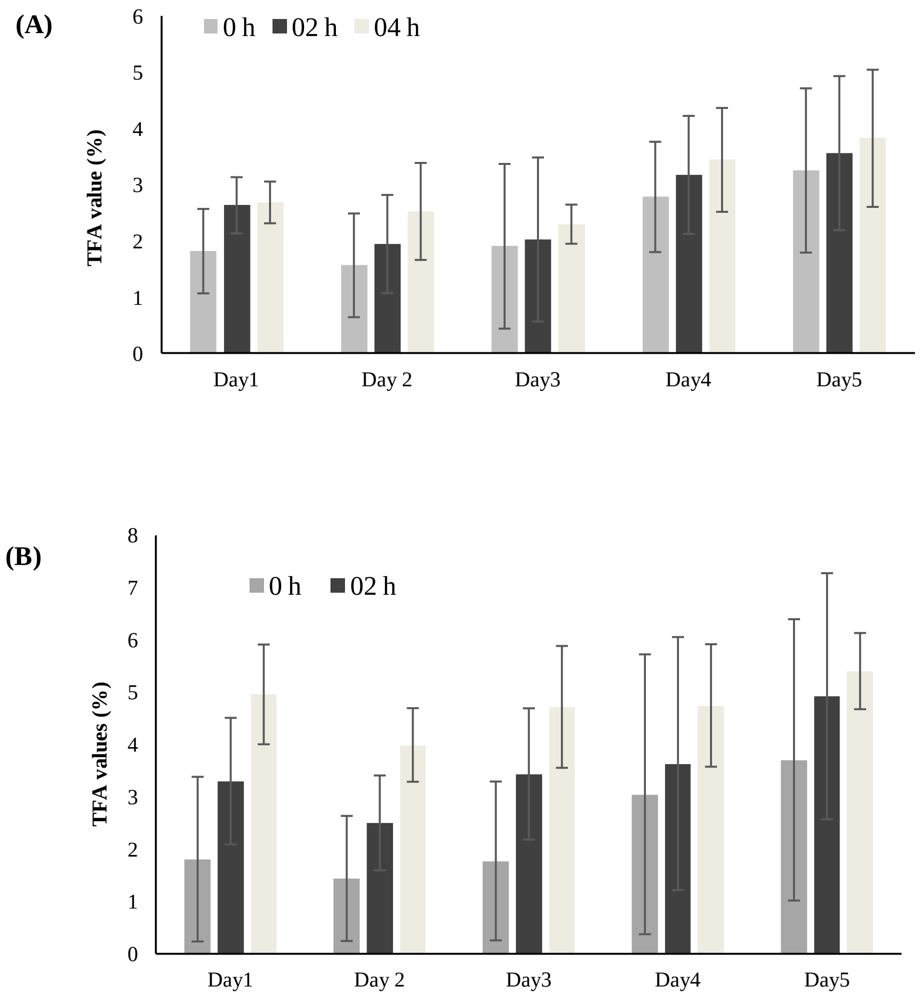


Figure 3: Mean Trans Fatty Acid (TFA) values of fried oil samples collected from Samosa (A) and Jalebi (B) vendors

A similar decreasing pattern at 2922 cm^{-1} was observed in an earlier study on palm oil frying (Poiana, 2012). Hashim et al. (2017) stated that there was a linear relationship between peak area at 2922 cm^{-1} with the peroxide value and conjugated Dience value. Therefore, decrease pattern at 2922 cm^{-1} (Figure 1A and 1B) with the frying time in this study may be correlated with the decrease in peroxide value as most of the hydroperoxides might have converted to secondary oxidation products (Zahir et al., 2017). A decrease in the absorbance peak at 1745 and 1654 cm^{-1} with increase in frying time could be due to the hydrolysis of triglyceride ester linkage and triglyceride fatty acid cis double bond (Goburdhun et al., 2001; Poiana, 2012). Hydrolysis of triglyceride and decrease in polyunsaturated fatty acids content was also reported in the previous study conducted on palm oil (Hashim et al., 2017). Changes observed at 966 cm^{-1} with the increase of frying time of processed edible oils reflects the trans double bond C-H bending vibration (Sherazi et al., 2009). This also indicates the development of TFA during the heating of all these oil samples. Previous studies (Goburdhun et al., 2001) reported that frying of C18: 1 and C18: 2 rich soybean oil resulted in the formation of TFAs.

Similar to the findings of the present study, FTIR spectral analysis of unheated and repeated heating soybean oil revealed a decreased peak absorbance at 1163 cm^{-1} for the oil heated for 40 and 60 min duration at constant temperature of 200 °C when compared to unheated oil (Ma et al., 2014). Decreased peak absorbance at 721 cm^{-1} with the frying time (Figure 1) may be related to the loss of the cis conformation double bond, i.e. unsaturation in the oil with increase in frying time (Poiana et al., 2015).

Similar to our results, previous studies by Karn et al. (2013) analyzed the oil samples used in preparation of Indian fried items such as Balushahi, Mathari, Bhatura, Bread Pakora, and Samosa; the TFA% ranges of oil used in preparation of these five foods were 0.27-6, 3.4-7.2, 4-23.8, 1.07-15.3, and 1.18-9.12%, respectively. In a survey carried out by Agrawal et al. (2008), analysis of 150 different Indian sweets and snacks revealed that TFA content of sweets varied from 0.3 to 17.7% and snacks from 0.1 to 19.8%. These researchers stated that some snacks like Samosa and Jalebi are the major contributor for TFA intake by Indian consumers. The main reason for TFA contribution by these foods is the hidden ingredients like flour, Bengal gram flour, hydrogenated fats, sugar, etc. Another study on 280 commercial Spanish foods showed high TFA values in French fries (20.9%) and microwave popcorn (46%), while relatively low values of TFA varied from 1.8% in biscuits to 4.6% in donuts (Fernández and Juan, 2000). The TFA composition analysis in several bakery products in Lebanon was also found to have the TFA in the range of 0.7 to 25.8% (Saadeh et al., 2015). Similar work revealed that TFA concentration in Brazilian biscuits and salty snacks were 0.86 and 7.94%, respectively (Dias et al., 2015).

The literature data indicate that hydrogenated vegetable oil plays a significant role in formation of TFA. In the present study, increased TFA levels were found in Jalebi fried oils and this might be due to the use of hydrogenated vegetable oils used by the Jalebi vendors. An increase in TFA of Samosa and Jalebi fried oils with increase in duration of frying was in line with the findings of Sanibal and Mancini Filho (2004). Higher TFA generation in Jalebi could be due to this fact that the high moisture content could accelerate the hydrolysis reaction during frying process which was earlier observed by Mishra and Sharma (2014).

Conclusion

The qualitative analysis of frying oil samples from Indian street vendors indicated that increase in frying time decreased the peroxide values and increased saturated fatty acids and TFA values of oils used for both the food items. On quantitative analysis of ATR-FTIR spectra, the TFA values of 4 and 17.4% of fried oil samples used for frying the Samosa and Jalebi were respectively found to be higher than the allowable limit. The local vendors and consumers should be educated by regulatory authorities regarding health risk of TFA in street fried snacks.

Author contributions

R.K. did the conceptualization, investigation, formal analysis, methodology, validation, visualization, and manuscript writing. B.B. and T.A. wrote the manuscript and supervised the study. S.C. did the conceptualization, methodology, validation, resources, manuscript writing, project administration, and supervision.

Conflicts of interest

The authors declare no conflicts of interest.

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