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Estimation Of Free Fatty Acids, Peroxide Value And Trans Fatty Acids In Reheated And Reused Oils Of Deep Fat Fried Street Foods

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ABSTRACT

Background: In India street foods are not only imparting the taste, but also forming a big sector in the society as a less expensive food source. Hence it is important to ensure the effects of oils used by street food vendors on the consumer health. As a major concern, Free Fatty Acids, Peroxide Value and Trans fats in reused oil is estimated to check the safety for the consumption of these street foods.
Objective: The objective of the study was to estimate Free fatty acids, Peroxide value and Trans fatty acids in reused and reheated oils and to compare with global and Indian standards.
Materials and methods: Reheated and reused oil samples were pooled from 6 areas of Vishakhapatnam, AP, India and estimation of Free Fatty Acids, Peroxide Value by titrimetry and Trans fats were estimated by extraction of fat, methylation and gas chromatography method.
Results: Randomly pooled deep fat fried oil samples from street vendors of Vishakapatnam city from selected areas showed Trans fatty acids <1.0% for all samples. However the fresh oil sample from PDS shown highest value which is nearer to <1.0% i e., 0.93%. The standards compared for estimation were fatty acid methyl esters.
Conclusion: Rearrangement of cis- trans isomers occurs during reheating and reusing the oils which lead to increase in the Trans fatty acids in oils. According to the WHO standards, TFA intake should be less than 1% of the total energy intake. Hence more strict legislative measures are required, as Trans fats have shown ill effects on health. In view of a public health concern issue, it is important to have proper standards for street foods especially with respect to oils and consumer awareness through labeling will help to reduce the risk of cardiovascular diseases.
Key words: Trans fatty acids, Street Food, Reused and reheated oils, Food safety.

Introduction

Lipids are one of the major constituents of foods and they are the major source of energy. Lipids, fats and fatty acids are an integral part of the human diet responsible, in general for energy provision, growth and development (Chang & Chow, 2007). The high energy content of lipid and fat fraction make it a debatable component of the diet, often blamed for excess energy which results in obesity, and high blood cholesterol levels. It is important to note that not all, but a certain groups of fatty acids such as saturated fatty acids are responsible for weight gain, obesity and heart diseases (Haug, Hostmark, & Harstad, 2007). Fats and oils in foods are oxidized during processing and preservation. This reaction causes deterioration in taste, flavor, odor, color, texture and appearance and finally they decrease the nutritive value of foods. Furthermore this reaction can induce food poisoning. Therefore from a food quality and food safety perspective this oxidation reaction must be suppressed.
Peroxide value has its own importance in assessing the food quality and food safety. The extent of purity of an oil or fat can be estimated by the peroxide value. It is also useful in predicting the shelf life of oil which is used regularly in every domestic need. Free fatty acids (FFA) are included amongst compounds naturally present in low amounts in vegetable oils. Being a product of hydrolytic degradation of triglycerides, their amount is considered as an important quality index for

oils, so that threshold values of FFA content in vegetable oils are provided by regulations and they may vary according to the oil type and the commercial class. The free fatty acid content is known as acid number/acid value. Normally, fatty acids are found in the triglyceride form, however during processing the fatty acids may get hydrolyzed into free fatty acid. The higher the acid value found, the higher the level of free fatty acids which translates into decreased oil quality. The keeping quality of oil therefore relies upon the free fatty acid content. It will measure any acid present in the food and extracted with the organic solvent used. This will include acids that may have been added to the food or produced during storage by fermentation or microbiological spoilage, therefore the FFA of a fresh product needs to be known always.

Trans fat is common name for unsaturated fat with trans isomer (E- isomer) fatty acids. Because the term refers to the configuration of double carbon-carbon bond, trans fats may be monounsaturated or polyunsaturated but never saturated (IFST, 2007). Trans isomers has higher melting points. These melting characteristics made it possible to produce desirable properties for plastic shortening by hydrogenation. Salad oil contains about 8-17 %, shortenings contains 14-60% and margarines around 70% of trans fats (Spiller, 1996). Main fatty acids produced from trans fats are oleic, elaidic, stearic acids. In dairy and meat products, generally the trans fats are 2-5% of the total fat content. TFA level of the total fat content in vanaspati could be as high as 50-60 % (FSSAI, 2013). The major consumption of TFA is through the industrially produced items such as cookies, cakes, margarine, French fries, and other fried products. The naturally occurring trans fats are produced in the gut of the animals, which comes in milk and meat.

Trans fats are not essential fatty acids, indeed the consumption of trans fats increases the risk of coronary heart disease by raising levels of "bad" LDL cholesterol and lowering levels of "good" HDL cholesterol (Innis *et al.,* 1999; Stender and Dyerberg 2003). Health authorities worldwide recommend that consumption of trans fat be reduced to trace amounts. Trans fats from partially hydrogenated oils are more harmful than naturally occurring oils. At 7% intake, trans fats tend to increase triglycerides and lipoprotein-α which are atherogenic and thrombogenic. Platelet aggregation attributed to thrombosis also increases due to high intake of Trans fats (Spiller, 1996).

Increased Trans fats consumption also showed association with increased risk of heart disease, cancer, diabetes, allergic conditions and effect on fetus (Sapana and Nirmali, 2009). When they compared, contribution of TFA for ischemic heart disease is quite more than saturated fat (Stender *et al.,* 2004), and also positive association was found between TFA and incidence of cancer of breast and intestine (Bakker *et al.,* 1997). In the past 5 y a series of metabolic studies has provided unequivocal evidence that trans fatty acids increase plasma concentrations of low-density-lipoprotein cholesterol and reduce concentrations of high-density-lipoprotein (HDL) cholesterol relative to the parent natural fat. In these same studies, trans fatty acids increased the plasma ratio of total to HDL cholesterol nearly twofold compared with saturated fats (Ascherio and Willet, 1997). The research data collected by Salmeron *et al.,* (2001) suggested that there is association of increase risk of type 2 diabetes with TFA. WHO has recommended that 1per cent energy limits to be derived from Trans fats. As per FSSAI standards <10 % of TFA are allowed in oils and vanaspati. But if 10 per cent Trans fatty acid level is allowed in vanaspati and other oils, more percentage of energy will be derived from trans fats, causes harmful effects and which are not recommended (Sapana and Nirmali, 2009). NIN has, focused on the melting point regulation, which is in line with codex Alimentarius guidelines. The melting point regulation for vanaspati i.e. between 31- 40 should be removed. FSSAI along with NIN made mandatory labeling regulations for TFA and SFA content on vanaspati packs, edible oils or any other product containing TFA from vanaspati source. According to the NIN risk assessment report on Trans fatty acids in the Indian diets, revealed that the fat consumption in rural and urban area is 20 and 30 g/day respectively.

According to the CSE (Centre for science and environment) lab report, it was found that 7 leading vanaspati brands in India showed trans fats 5-12 times the 2 per cent standard set by Denmark. Keeping this in view, the health ministry in 2008 came out with a notification for labeling Trans fats in foods without a standard. International and national standards for consumption of Trans fats in different countries are stated in table 1 and 2.

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Table 1 International standards for Trans fat

Country / organization	Standards
WHO	TFA <1% of total daily energy intake
Denmark	<2 % TFA permitted in oils and fats <1 % in foods represent as free of trans fats
Canada	TFA limits to 2% of total fat content of oils and margarine

Table 2 National standards for Trans fat

Organization	Standards
FSSAI	<5-10 % of trans fats in PHVO and FATS
Prevention of food adulteration act-1954	Information on package about SFA, MUFA, PUFA, Trans Fats
AGMARK	NO specific standards given

Street foods are ready to eat food, which include many types ranging from cereal and fruits to cooked meat and drinks. Studies have shown that street vendors have always played an important role in the supply of food in urban areas. It has also been an important source of employment, especially for women and youth. Changing lifestyles and family structures have meant changing eating habits and this has led to increase popularity of street food (Street food safety report, Vishakhapatnam, 2008).
According to Food and Agriculture Organization (2007), 2.5 billion people eat street food every day. In addition to being significant source of food for urban dweller, street food has also been engaged as tourist attraction. With the increasing pace of globalization and tourism, there is a need to evaluate the Trans fats present in the reheated oil or reused oil in the street food. Safety and hygienic condition of the street food vendors has become one of the major concerns of public health. World bank mission has already carried out a street food survey project in 16 cities of India to check the present status of street food in different cities (CBP Newsletter, 2006). Such kind of projects is the need of present food safety issues.
India is the third largest consumer of oil (Sapana and Nirmali, 2009). Palm oil is used in various forms and also used for making vanaspati which is a hydrogenated fat. In India palm oil is used by many small food business operators. Hence testing and regulation of the oil is very important to control the TFA percentage in palm oil and other oils which are largely used by the consumer. The present research study has been done on the reused and reheated oil samples to estimate the presence of Free fatty acids, Peroxide value and Trans fats in the oils samples collected from street vendors.

Materials and Methods:

The researcher has selected and interviewed 118 street vendors across Vishakhapatnam city, which were divided into 6 areas according to street food vendor distribution and eating pattern of the population. All street vendors were `interviewed about their basic education, selling of the deep fried food item and type of oil they are using for frying. From volunteered street food vendors of each area, the oil samples were collected and pooled. Finally 6 samples were analyzed for TFA corresponding to each area. Because of its cost effectiveness 100 % of the street vendors were using Palm oil for deep frying of their street foods. Initially fresh oil samples were analyzed for its free fatty acid (FFA) content and peroxide values then compared with used samples and Standards. Estimation analysis of FFA and peroxide value was followed according to Ranganna, (1986) which is titrimetric method.

Determination of Fat (by Acid Hydrolysis capillary Gas Chromatographic Method)

Principle: Total Lipid extract is obtained by digesting test portion with hot HCl. Hydrolyzed fat components are extracted into ethyl and petroleum ether, and evaporated. The extract is saponified and methylated. Fatty acid methyl esters are determined by capillary Gas Chromatography. Total Fat is calculated as sum of individual fatty acids expressed as triglycerides equivalents. Saturated, Unsaturated, and mono unsaturated fats are calculated as sum of individual fatty acids. The Method can be used for quantification of Trans isomers when using appropriate standards of Fatty acid methyl esters.

Procedure: There are 3 steps involved in determination of Fatty acids.

Step-1: Extraction of Fat

Test portion (W) accurately weighed 2 g and taken into extraction flask. While shaking 2 ml ethanol was added to ensure wetting of all particles. Then 10 ml 8M HCL was added and was covered with screw cap. Contents were mixed for 5 sec until thoroughly moistened. For additionally 5 seconds it was mixed on vortex mixer. Then the tube was placed in water bath at 80°C and was hold for 40 min..

Then the tube was removed from water bath and 10 ml ethanol was added immediately. After mixing for 5 seconds on vortex, it was cooled to ambient temperature. Ethyl ether 30 ml, was added to it and tube was shake by partial inversions. Vent pressure often by removing stopper. Then 30 ml petroleum ether was added and it was shacked for 1 min.

The two layers were separated after some time. The ether layer was decanted from the tube and poured into pre weighed (W1) 250 ml flat-bottom flask through funnel containing fat free cotton or glass wool. The extraction of fat was repeated twice using 15 ml of ethyl ether and 15 ml petroleum ether, with shaking well after each addition of ether and then filtered. Slowly evaporated the ether layer under steam table of dry N. The final weight (W2) was taken of flat bottom flask and calculated the % of the Fat content using following formula.

% of Fat = (W2 - W1) * 100 / W

Step-2: Methylation

Methanolic NaOH solution 10 ml, was added to extracted fat. Then flask was attached to water cooler condenser, joint was sealed with methanol and heated. It was reflux for 10 min. then 10 ml BF3reagent was added from the top of the condenser. Continued the reflux for additional 5 min. Added 10ml n-Heptane through the top of condenser and reflux additional 1 min.

Flask was removed from the heat and transferred the entire mixture into centrifuge tube. The flat bottom flask was rinsed with 5 ml saturated NaCl solution. Contents of tube were mixed by shaking gently and layers were separated. Upper layer contained Fatty acid methyl esters in n-Heptane. Transferd 1ml of upper layer to GC vail and used for analysis.

Step-3: GC determination

Injected 1µl FAMEs standard solution of known concentration onto GC Column using following conditions:

Conditions for GC: Injector Temperature: 250°C**.,** Detector Temperature: 280°C**,** Split Ratio:10

Column Oven conditions

Temp(°C)	**Rate**	**Hold(Min)**	**Total(Min)**
120	-	1	1
175	10	10	16.5
210	5	5	28.5
230	5	5	37.5

Makeup gas flow: 25.0ml/min, H2 gas Flow: 30ml/min, Air Flow: 300ml/min, N2 gas flow through column: 2.0ml/min,1 µl of test solution was injected into GC column. The corresponding fatty acids were calculated using area normalization basis.

Calculations:

Saturated fat (%) = ΣSFA a * Fat (%) / 100

Unsaturated fat(%) = ΣUFA a * Fat (%) / 100

Monounsaturated fat(%) = ΣMUFA

Where SFAa = Area of all saturated fatty acids

FAa = Area of all fatty acids

UFa = Area of all unsaturated fatty acids

MUFAa = Area of all monounsaturated fatty acid

Results and Discussion:

The amount of free fatty acid present in food samples are affected by many parameters viz., moisture, light, air and temperature, because these parameters are reflecting the shelf stability of the final food products. Therefore in this study the commercial brands of oil was procured from Vizag market and they had been analyzed for their fatty acid profile after subjecting them to an elevated temperature

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ranging from 60˚C to 220˚C for 5 mts. The results (table no 3-7) showed that the olive oil and groundnut oil contributed to significant reduction in free fatty acid value of 0.28% & 5.32%, from the initial value of 0.84 and 9.81%, respectively. The fatty acid profile for this oil viz., palmitic acid, oleic acid, lauric acid and ricinoleic acid were also followed the similar pattern of reduction at these elevated temperatures. While other oils like coconut oil, palmolein oil, rice bran oil and gingelly oil doesn't exhibited wider variation in terms of acid value and also individual free fatty acid value at above conditions. Among the various fresh samples analyzed the rice bran oil exhibited lesser acid value of 0.1% followed by olive oil of 0.42%, coconut oil of 0.8%, palmolein oil of 1.41%, gingelly oil of 3.6% and groundnut oil of 4.93%. Therefore on the account of residual trace account of free fatty acids presence, it is advisable to use rice bran oil and olive oil for cooking purpose when compared to other oils that are available in market.

Table no 3: Acid values of different fresh oils at increasing temperatures

S no	Name of the oil	30^0 C	60^0 C	100^0 C	140^0 C	180^0 C	220^0 C
1.	Olive	0.84	1.96	1.40	1.12	0.84	0.28
2.	Coconut	1.6	0.84	1.1	1.1	0.28	0.84
3.	Palmolein	2.8	1.12	1.68	0.56	1.12	1.12
4.	Groundnut	9.81	8.69	8.13	7.57	6.45	5.32
5.	Rice bran	1.96	1.40	0.84	0.84	1.12	1.12
6.	Gingelly	7.29	5.6	5.6	5.6	6.4	4.7

Table no 4: Free Fatty Acid values in terms of Oleic acid of different fresh oils at increasing temperatures

S no	Name of the oil	30^0 C	60^0 C	100^0 C	140^0 C	180^0 C	220^0 C
1.	Olive	0.42	0.99	0.71	0.56	0.42	0.14
2.	Coconut	0.8	0.4	0.5	0.5	0.14	0.4
3.	Palmolein	1.41	0.56	0.84	0.28	0.56	0.56
4.	Groundnut	4.93	4.37	4.08	3.80	3.24	2.67
5.	Rice bran	0.1	0.7	0.3	0.3	0.4	0.4
6.	Gingelly	3.6	2.9	2.9	2.9	3.2	2.3

Table no 5: Free Fatty Acid values in terms of Lauric acid of different fresh oils at increasing temperatures

S no	Name of the oil	30^0 C	60^0 C	100^0 C	140^0 C	180^0 C	220^0 C
1.	Olive	0.3	0.7	0.5	0.4	0.3	0.1
2.	Coconut	0.6	0.3	0.4	0.4	0.1	0.3
3.	Palmolein	1.0	0.4	0.6	0.2	0.4	0.4
4.	Groundnut	3.5	3.1	2.9	2.7	2.3	1.9
5.	Rice bran	0.7	0.5	0.3	0.3	0.4	0.4
6.	Gingelly	2.6	1.9	1.9	1.9	2.3	1.7

Table no 6: Free Fatty Acid values in terms of Palmitic acid of different fresh oils at increasing temperatures

S no	Name of the oil	30^0 C	60^0 C	100^0 C	140^0 C	180^0 C	220^0 C
1.	Olive	0.38	0.90	0.64	0.51	0.38	0.13
2.	Coconut	0.7	0.3	0.5	0.5	0.12	0.3
3.	Palmolein	1.28	0.51	0.77	0.26	0.51	0.51
4.	Groundnut	4.48	3.96	3.71	3.45	2.94	2.43
5.	Rice bran	0.89	0.64	0.38	0.38	0.51	0.51
6.	Gingelly	3.3	2.4	2.4	2.4	2.9	2.1

Table no 7:Free Fatty Acid values in terms of Ricinloeic Acid of different oils at increasing temperatures

S no	Name of the oil	30^0 C	60^0 C	100^0 C	140^0 C	180^0 C	220^0 C
1.	Olive	0.45	1.04	0.75	0.6	0.45	0.5
2.	Coconut	0.8	0.4	0.5	0.5	0.14	0.4
3.	Palmolein	1.49	0.6	0.8	0.3	0.6	0.6
4.	Groundnut	5.21	4.61	4.32	4.02	3.42	2.83
5.	Rice bran	1.04	0.74	0.44	0.44	0.59	0.59
6.	Gingelly	3.87	2.8	2.8	2.8	3.4	2.5

Detection of peroxide value gives the initial evidence of rancidity in unsaturated fats and oils. The double bonds found in fats and oils play a major role in autooxidation. Peroxides are the intermediates in the autooxidation reaction. Auto oxidation is a free radical reaction involving oxygen that leads to deterioration of fats and oils which form off flavors and off odors. This value is determined by measuring the amount of iodine which is formed by reaction of peroxides with iodine ion. Peroxide value of fresh oils (table no 8) is less than 10 milliequivalents/kg, when it is between 30 & 40 the rancid taste is noticeable. Therefore it is essential to ascertain the edible oils to be free from rancidity. In this study the various commercial brand oils had been analyzed to determine its peroxide value. The analysis from table no 8 showed that the palmolein oil and olive oil recorded peroxide value of 2 milliequivalents/kg; groundnut oil recorded 3 mil liequivalents/kg, sunflower oil recorded 1 milliequivalents/kg, rice bran oil recorded 4 milliequivalents/kg, ghee and gingelly oil recorded nil peroxide value.

Therefore the commercial oil brand samples which are available in Vizag market which shows that all samples are found to be within the prescribed limit of peroxide value and these oils are fresh and they can be utilized for cooking purpose without prone to any oxidative rancidity.

Table no 8: Peroxide values of fresh commercial oils

S no	Name of the Oil	Peroxide Value
1	Gingelly	0
2	Coconut	0
3	Rice Bran	4
4	Palmolein	2
5	Sunflower	1
6	Vanaspati	3
7	Groundnut	3
8	Olive oil	2
9	Ghee	0

The chemical changes which occur can be of two types of reactions: hydrolysis and auto-oxidation (Gregory and Andrade, 2004) . Hydrolytic reactions are catalyzed by the action of heat and humidity, with the formation of free fatty acids, monoacyl glycerol and diacylglycerol. The lipid auto-oxidation is associated with the reaction of oxygen with unsaturated fatty acids and occurs in three stages: initiation, propagation and termination (Ramalho and George, 2006). In initiation, they are free radicals formed from the removal of a hydrogen from carbon fatty acid molecule, in favorable conditions for light and heat. The spread is the step in which free radicals are readily susceptible to atmospheric attack, are converted into other radicals, forming peroxides and hydroperoxides, conjugated dienes, hydroxides and ketones, which are the primary oxidation products (Borgo and Araujo, 2005; Ramalho and George 2006) and can decompose in small pieces or remain in the molecule of dimeric and polymeric triacylglycerols. Thus, the last step of the oxidation process is the termination, where two radicals forming stable products obtained by scission and rearrangement of peroxides (Fox and Stachowaik 2007).

Six areas were selected for the study. A total of more than hundred i.e. 118 street vendors who are selling deep fat fried foods were enumerated. From the results (table no 9 and figure no 1) it was noticed that the Trans fats ranged from 0.10% to 0.93%. The highest Trans fat was seen in the sample of public distribution system. All the samples estimated the Trans fats were below < 1%. The results are expressed in table no. 5. The street vendors were working for an average 5-6 hours per day. For that they were keeping the oil on continues heating for about 4 hours on an average.

As the FSSAI and other national agencies are focusing on the standardization of Trans fats in oils and fats, it is very important to have measures to control the street food products containing more oil. Above results showed the effect of overheating and reusing on the oil. Hence, it is surely necessary to control trans fat percentage in hydrogenated fats and oils.

Table no 9:Trans fats in estimated oils

Sno	Area	Percent Trans fats
1	MVP	<0.1
2	Arian	<0.1
3	Collector office road	<0.1
4	Harbor area	<0.1
5	One town area	<0.1
6	Sample from PDS	<0.93

Representation of chromatograph of the oils

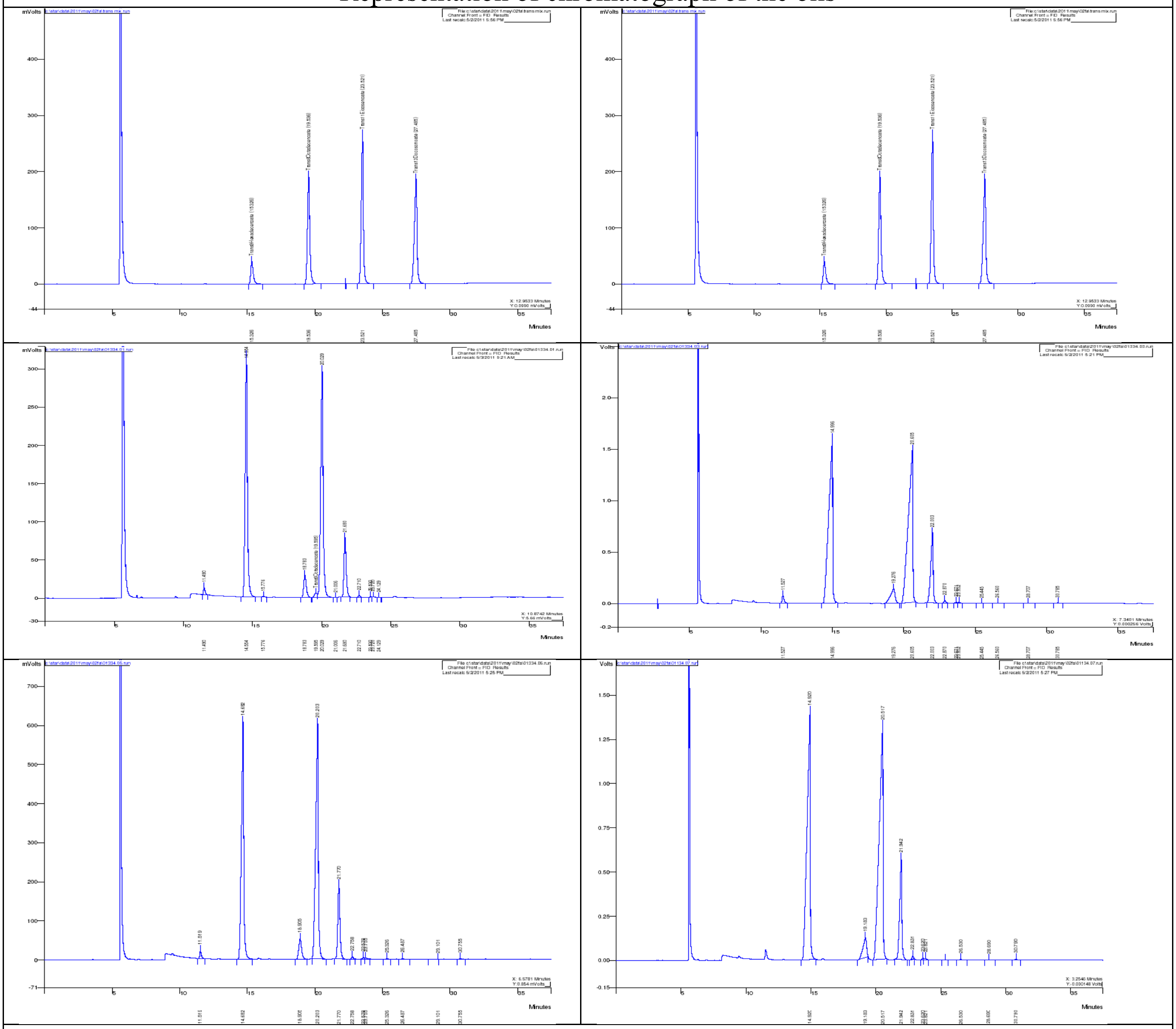

Fig no 1: Chromatograms of the estimated oil samples

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A study done by Downs *et al.,* (2015) in India found that the major source of dietary trans fat in the survey was from the consumption of commercially prepared snacks (i.e., consumed away from home). Thirty-one percent (n = 82) of households were consuming snacks containing high trans fat in villages as compared to 84.3 % (n = 220) in slums. The median total fat consumption was higher in the urban slum (36.9 g/CU/day) as compared to the rural villages (29.5 g/CU/day; $p < 0.001$) and a larger proportion of fat was attributed to snack consumption in the urban slum (median 10.8 g/CU/day, range 0–90.2 g/CU/day) than in the rural villages (median 1 g/ CU/day, range 0–58.5 g/CU/day; $p < 0.001$). The median trans fat consumption was 0.22 g/CU/day (range: 0.01- 14.79 g/CU/day) in the villages and 0.67 g/CU/day (range: 0.01-11.44 g/CU/day) in the slums ($p = 0.001$). The majority of the households did not exceed the WHO recommendation to limit trans fat intakes to less than 1 % of total energy; however, 4 % (n = 10) of village households and 13 % (n = 33) of urban households consumed more than 1 % of total energy from trans fat. According to Enig et al., (1990) based on weighted averages for the trans levels in each fats and oils category, the estimates of trans FAs available in the U.S. food supply range from 12.5 to 15.2 g/person/day (average 13.3 +/- 1.1 g/person/day).
The World Health Organization (WHO) recommends that trans fat virtually has to be eliminated from the global food supply. Although several high-income countries have successfully reduced trans fat levels in foods, however low- and middle-income countries such as India face additional challenges to its removal from the food supply. A multisectoral food chain approach to reducing trans fat is needed in India and likely in other low- and middle-income countries worldwide. This will require investment in development of competitively priced bakery shortenings and economic incentives for manufacturing foods using healthier oils (Downs *et al.,* 2015).
The removal of trans fat from the global food supply has been deemed one of the most straightforward public health interventions to reduce NCDs. LMICs such as India are likely to face additional challenges to trans fat reduction related to the large informal food sector which includes the small manufacturing enterprises and small traders and service providers, legal and illegal activities and a wide array of artisans. These include reduced capacity for enforcement, lack of coordination among policy sectors, competing health priorities and lack of awareness regarding trans fat (Leth *et al.,* 2006; Colón-Ramos *et al.,* 2007; Mozaffarian and Stampfer, 2010; Colón-Ramos *et al.,* 2014; Pérez-Ferrer *et al.,* 2015; FAO, 2015). Actions to reduce TFAs need to be carefully considered regarding both the intended and unintended consequences related to nutrition and public health (Filip *et al.,* 2010).

Conclusion and summary

In the future, it will become increasingly difficult to assure food security as well as food safety, and also the nutritional quality of food. Indeed, it is the nutritional quality of food and its distribution all over the world that are the main factors which will have a huge impact on human health. As per the above results and discussion we can conclude that the more the reheating and reusing of oil, the percentage of trans fats increases. As shown for PDS sample, oil heated for more than 4 hours on an average, has higher value compare to others due to continues heating of the oil. Hence we can state the street food should be checked from public health's point of view and effects of trans fats on the body, as the consumption of street food is high and increasing due to globalization and tourism. As FSSAI and other national institutes are already taking measures to control the TFA in industrially produce food items, it is a surely positive step to decrease the health risks associated with TFA. Further research can be done on the consumption of trans fats by large population through street foods when compare to recommended intake.

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