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Biochemical changes and fatty acid profiling of consecutively used fried groundnut oil

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Abstract
Groundnut oils have been shown to be a major source of fatty acids in human diets, additional standards and regulations are available in India but In India many food products are fried in groundnut oil and repetitively used. Therefore we have conducted experiments with groundnut oil with frying temperature of 150-165 ^{0}C and Fatty acids profiling of all three repetitively used oils were analyzed in GC-MS. Our results suggest that frying of groundnut oil showed characteristics pattern. The MUFA+PUFA/SFA ratio of the third, fourth and fifth stage of fried oil were 1.404, 0.898, and 0.753 respectively. It was decreased when consecutive frying oil reused. Similarly PUFA/SFA ratio was showed same trends and ranges between 0.379 to 0.462 in fifth and third oil. The MUFA/SFA was shown decreasing trends in third, fourth and fifth stage of fried oil. Thereby we suggest that groundnut oil is not suitable for deep-frying after third stage and at fifth stage it reaches up to 11%. There is currently legislation on Tran's fats levels in fried products of regulations on FSSAI. In addition to this our results indicated that for the food safety concerns, usage of groundnut oil in repeated use for cooking and fried must be avoided.

Keywords: biochemical, oil, groundnut, consecutively

Introduction
Fats and oils play important functional and sensory roles in food products. They are responsible for carrying, enhancing, and releasing the flavor of other ingredients, as well as for interacting with other ingredients to develop the texture and mouth-feel characteristics of fried foods. Groundnut is oil most often used in Chinese, South Asian and Southeast Asian cuisine. This oil is rich in high smoke point relative to other cooking oils. In addition, it has a pleasing taste and its major component fatty acids are oleic acid (MUFA), linoleic acid (PUFA), and palmitic acid (SFA). The oil also contains some stearic acid, arachidic acid, arachidonic acid, lignoceric acid and other fatty acids. This is one of the heart friendly oils which are not only rich in MUFA but otherwise also well balanced. As we know that vegetable oils have no cholesterol, but some of them can lead to endogenous (by the body) production of cholesterol. There are numerous references suggested that Trans fat produced due to deep frying or shallow frying and many scientist advocated that never used refried oil in food.

Frying is a very important method of cooking in the food industry as it enhances the sensory and physical properties of foods. Fried foods are desired for their distinctive flavor and odor. During frying, the oil is continuously and repeatedly used at elevated temperatures (160-180 °C) in the presence of air and moisture. When foods are fried in heated oil, many complex chemical reactions occur resulting in the production of degradation products. As these reactions precede, the functional, sensory and nutritional quality of the fat/oil changes and may eventually reach a point where it is no longer suitable for preparation of high quality fried products and the frying fat/oil will have to be discarded and therefore the risk factors associated with coronary heart disease and cholesterol levels correlate to the amount of fatty acids such as linoleic acid and other saturated and polyunsaturated fatty acids. (Logan, *et al.*, 1978) [5].

On the other hand, few studies have analyzed fatty acids contents found in cooked groundnut oils (e.g., deep-fried oils) despite the repeated use of fried oils especially in small restaurants and vendors. Therefore, it is important to determine the levels and compositions of fatty acids found in commonly used groundnut oils. The objective of this study is to analyze saturated, unsaturated poly unsaturated and certain trans fat found in fresh and cooked groundnut oils with five times refrying.

To achieve this objective, we examine groundnut oils commonly used in Gujarat and Saurashtra regions for frying of variety of various products of potatoes.

Experimental materials

Frying and sampling

Frying was performed by placing three potato chips time in groundnut oil in a 3 L deep fryer at 150 to 165 ^{0}C, 6 min 30 s or Fresh groundnut oil was used for all pre-frying and frying processes. Five frying operations were done and oil quality was measured. The remaining oil from the pan surface was collected by manually shaking the aluminium basked that was further used in next consecutive frying after 3 hours of cooling.

Sample analysis

Lipid extracts were obtained from 3 g of sample according to Gillan, F. T. (1983) [3] and slight variation in (Woodbury *et al*., 1995 and 1998) [12, 11]. Fatty acid composition was determined by GCMS. Boron trifuoride/methanol was used for the separation of fatty acid methyl esters (Morrison and Smith, 1964) [6], which were dissolved in hexane. Shimadzu series GC MS fitted with a capillary column DB-WAX (30 m0.25). i.d. and 0.25 mm film thickness) and a flame ionization detector was used. The temperatures of the injection port and the detector were 230 and 250 ^{0}C, respectively. The carrier gas was hydrogen, 1.7 ml /min. The use of GC column allowed to separation and identification of standard fatty acids, including some minor isomers. Samples were methylated after the cooking of potato chips and initially as well as repetitive five stages of repetition. The same day in duplicate and then analyzed on the DB - WAX 30-m column within the same run. The sample volume was 1 ml. Fatty acid methyl esters were prepared. For Detection of fatty acids by comparison of the retention times of the peaks in the sample with those of standard library used for a fatty acid internal standard. Fatty acid content was expressed as percentage of total fat content. The common name was identified with the use of chemspinder and bioinformatics tool for lipids and used in standards.

Free fatty acid: Fatty acid concentrations were determined by titrimetry by AOAC Official method 993.23 and acid value is defined as the number of mg of KOH required to neutralize the fatty acids contained in 1 g.

Saponification value

The all consecutive fried oils were used for determination of saponification value as procedure described in AOAC Official method 920.16. Saponification value indicates the average molecular weight of a fat or oil. Weigh 1 gm of oil, Add 20 ml of 0.5 N alcoholic KOH solutions to the round bottomed flask. Follow the above procedure without taking oil for blank titration. Reflux both round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate both the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

Peroxide value

The peroxide value (PV) test, which is one of the most common tests used to evaluate the extent of lipid oxidation, is based on measuring peroxides as adopted by AOAC Official method 965.33. Measure off 10 ml of 0.01N $K_2Cr_2O_7$ solution to a 200 ml conical flask. Add 0.5 ml concentrated HCl and 1.0 ml 15% KI solution. Mixed exactly 1 minute and leave for 5 minutes in a dark place. Add 0.5 ml starch solution, 20 ml distilled water. Mix and titrate with sodium thiosufate solution. Calculate the exact normality of $Na_2S_2O_3$ knowing that in this chemical reaction 1 gram-equivalent of $K_2Cr_2O_7$ react with 1 gram-equivalent of $Na_2S_2O_3$ (1 mole $K_2Cr_2O_7$react with 6 moles $Na_2S_2O_3$).

Rancidity

Rancidity of experimental different cooking oils at repetitive deep frying of potato chips were measured by ranciate 743 (metrohm). Before the sample is weigh Care was taken for the sample, for not over heated and not contaminated by any other materials during the experimentation. The parameters for sample size were liquid samples 3±0.1 g, measuring solution 60 ml, Temperature 80-160 ^{0}C, Gas flow 20 L / h and evolution sensitivity were 1.0 with induction time.

Results and Discussions

Biochemical characteristics of fried oil

Characteristics of groundnut oils at different stages of fried oil with biochemical analysis was presented in table 1. The peroxide values were increased after frying and it was ranges between 06 to 1.6 (Shantha and Decker 1994) [9] also determines value for this. Same was true for Saponification value, free fatty acids, Iodine value and smoke point. Peroxide value were doubled after first frying and then retain increasing trends in repeated use of fried oil. Same trends were observed in Saponification value, but almost 10 time's higher value than the before frying oil. Means saponification value increased at any stage of frying.

Free fatty acids is the important parameters for shelf life of any products, therefore during consecutive use of fried oil showed intense increased after third frying therefore data for fatty acids were presented from third frying to fifth frying oil. but one thing is noted that if someone utilized repeated uses of oils than it requires higher frying temperatures for frying and ultimately leads to increase in consumption of energy and maximum oil absorption in food materials therefore it is not advisable to use up to third stage of frying materials than it can cause highest effect on health of food and human beings too. Similarly iodine value were increased from 85 to 95 from first to fifth frying oil. Frying temperatures and Oil absorbed in fried products (gm) were increased up to fifth stage. The descriptive statistics of characteristics of all five oils were presented in table 2. Our results suggested that Mean, S.Em, Median, SD, Variance, Kurtosis, Skewness of FFA data were 0.09, 0.03, 0.09, 0.07, and 0.01. -1.72 and 0.44 respectively. Similar value for Peroxide value, Saponification value (mg KOH/gm), Iodine value, Smoke point, Frying Temp(C) and Oil absorbed in fried products were depicted in table.2.

Fatty acids

A typical chromatogram of groundnut oil of 3rd, 4th and 5th repetitions showed wider variation in number of fatty acids composition. As shown in table 3, 4 and 5, At third oil after frying showed initially 14 fatty acids and latter on, it was detected 17 in 4th stage and 30 fatty acid molecules in fifth oils means almost doubled than the third oil collected after frying of potato chips. The results from these experiments showed in common fatty acids exclusively one common i.e. Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester in third fried oil and fourth fried oil (Figure 1-4). While in 4th and 5th fried oil five common fatty acids were observed viz., Methyl tetradecanoate, Tetracosanoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, 9, 12, 15-

Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-, 13-Docosenoic acid, methyl ester, (Z), Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester. In comparison with third and fifth fried oil, there were three fatty acids were common, Octanoic acid, methyl ester, 7-Hexadecenoic acid, methyl ester, Octadecanoic acid, methyl ester. As shown in vendiagramme seven common fatty acids were presents in all three frying oils, they were Hexadecanoic acid, methyl ester, 9-Hexadecenoic acid, methyl ester, Heptadecanoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Eicosanoic acid, methyl ester, 11-Eicosenoic acid, methyl ester and Docosanoic acid, methyl ester. Third oil showed maximum area for 9-Octadecenoic acid, methyl ester, (Z) (33.22%), 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester (18.88%) and lower value was observed in Octanoic acid, methyl ester (0.07). Similarly in 4th oil after frying both fatty acids showed decrement in the value. The results showed decreased in 9-Octadecenoic acid, methyl ester, (Z) (17.84) and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (17.8) Where as lower contents of Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester (0.09%) was observed. According to pubchem data base it was found in human, certain bacteria and plants to Cyclopropane FA contain three-carbon carbocyclic rings located at different sites of FA chain. They have been also found in plants, bacteria, parasites, sponges and Ascidia (Carballeira, *et al*., 2007 and Yu, *et al*., 2011) [1, 13]. At fifth oil, 9-Octadecenoic acid, methyl ester, (Z) and 9,12-Octadecadienoic acid convert in to trans fat 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) and PUFA (9,12-Octadecadienoic acid (Z,Z)-, methyl ester) value were remained almost same but it was decreased about 50% after frying from third consecutive oil convert to fifth oil. Similar finding showed by many scientist but they had not observed the trends of particular fatty acids or comparison of individual fatty acids in fried oil. Our results suggested that it is advisable to use oil up to third repetition of oil for certain food products but in fifth stage it was drastically increased up to 11%. Means it is highly unhealthy for food. Therefore we advocate do not used groundnut fried oil highly become trans if repeatedly used of oil. Table 2 showed the composition and retention time of the components heated groundnut oil. Caproic acid, caprylic acid, nonanoic acid are saturated fatty acids whose quantities have changed upon heating, in the subsequent frying illustrating that they do not take part in any chemical reactions. The unsaturated fatty acids oleic acid and linoleic acid, as well as palmitic methyl ester, react strongly upon heating to increase the percentage of the saturated fatty acids stearic acid and arachidic acid. Similar findings were also measured by (Sathianathan *et al.*, 2014) [8].

Effect of frying on saturated and unsaturated fatty acids

Each triglyceride can be broken down into three fatty acids plus glycerol. All fatty acid from a triglyceride is classified as a saturated (SFA), a monounsaturated (MUFA) or a polyunsaturated (PUFA) fatty acid. Triglycerides are mixtures of the three different types of fatty acids, SFA, MUFA and PUFA; the proportion of each one determines the distinctiveness of fats in food and their effect on human health. As shown in table 6 the third oil showed degree of saturation increased in repetitive used of oil from 41.59 to 52.7 and degree of unsaturation were decreased from 58.41 to 49.25%. Similarly in MUFA (Mono unsaturated fatty acids) it was decreased 39.2 to 18.97. These results are in agreement with the study of Fatty acid composition of raw soybeans changed after the frying process. Palmitic, linoleic, linolenic acids, iodine value and saturated / unsaturated ratio decreased while oleic acid and the oleic / linoleic ratio (O/L) increased after the frying process of soybeans (Jauregui, *et al*., 2012) [4]. It was remain same in all stages of repeated used of oils but it convert to trans also. Our results of frying of groundnut oil showed characteristics pattern in fried oil. The MUFA+PUFA/SFA ratio of the 3rd, 4th and 5th fried oil were 1.404, 0.898, and 0.753 respectively. It was decreased when consecutive frying oil reused. Similarly PUFA/SFA ratio was showed same trends and ranges between 0.379 to 0.462 in fifth and third oil. The MUFA/SFA was shown decreasing trends in 3rd, 4th and 5th fried oil. These results are in agreement with Wang *et al.*, (2014) [10] study was used to explore the effect of frying time on the fatty acids content in soybean oil, peanut oil and sesame oil and suggested that total content of SFA were increasing trend in oil, while the total content of UFA all showed a trend of decline with a prolonged frying time. The trends of above ratio are in agreements with results were obtained by (Noor lida *et al*., 2002 and Christopoulou *et al*., 2004) [2, 7].

Table 1: Effect of repeated frying on Characteristics of groundnut oils

Times	Peroxide value	Saponification (mg koH/gm)	FFA (%)	Iodine value	Smoke point	Frying temperatures	Oil absorbed in fried products (%)
Stage-1	0.6	4.54	0.023	85	200	150	14.4
Stage-2	1.2	8.46	0.023	85	200	150	15.2
Stage-3	1.3	15.59	0.085	88	200	160	13.97
Stage-4	1.4	30.25	0.141	89	199	160	14.55
Stage-5	1.6	45.06	0.194	92	199	165	46.21

Table 2: Descriptive statistics of the fried groundnut oil

	Peroxide value	Saponification (mg koH/gm)	FFA (%)	Iodine value	Smoke point	Frying Temp. (^{0}C)	Oil absorbed in fried products (%)
Mean	1.22	20.78	0.09	87.80	199.6	157.0	20.9
S.Em	0.17	7.49	0.03	1.32	0.2	3.0	6.3
Median	1.30	15.59	0.09	88.00	200.0	160.0	14.6
SD	0.38	16.75	0.07	2.95	0.5	6.7	14.2
Variance	0.14	280.41	0.01	8.70	0.3	45.0	200.9
Kurtosis	2.52	-0.86	-1.72	-0.80	-3.3	-2.4	5.0
Skewness	-1.38	0.79	0.44	0.52	-0.6	-0.2	2.2
Min	0.60	4.54	0.02	85.00	199.0	150.0	14.0
Maxi	1.60	45.06	0.19	92.00	200.0	165.0	46.2

Table 3: Fatty acids profiling of fried groundnut oil in third consecutive stage

Peak No.	Retention time	Name of the compound	Type of Fatty Acid	Area %	common name
1	5.773	Octanoic acid, methyl ester	Saturated	0.07	Caprylic acid
2	14.289	Pentadecanoic acid, 14-methyl-, methyl ester	Saturated	0.15	Pentadecylic acid
3	18.015	Hexadecanoic acid, methyl ester	Saturated	15.18	palmitic acid
4	18.304	7-Hexadecenoic acid, methyl ester, (Z)-	MUFA	0.26	
5	18.419	9-Hexadecenoic acid, methyl ester, (Z)-	MUFA	0.52	Palmitoleic acid
6	19.759	Heptadecanoic acid, methyl ester	Saturated	0.51	Margaric acid
7	20.158	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	Saturated	0.31	Caprylic acid
8	21.745	Octadecanoic acid, methyl ester	Saturated	12.7	Stearic acid
9	21.958	9-Octadecenoic acid, methyl ester, (Z)-	MUFA	33.22	Oleic acid
10	22.786	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	PUFA	18.88	Linoleic acid
11	23.836	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	PUFA	0.33	Alpha linolenic acid
12	25.015	Eicosanoic acid, methyl ester	Saturated	5.71	Arachidic acid
13	25.35	11-Eicosenoic acid, methyl ester	MUFA	5.2	Gondoic acid
14	28.479	Docosanoic acid, methyl ester	Saturated	6.96	Behenic acid

Table 4: Fatty acids profiling of fried groundnut oil in fourth consecutive stage

Peak No.	Retention time	Name of the compound	Type of Fatty Acid	Area %	common name
1	14.298	Methyl tetradecanoate	Saturated	0.27	Myristic acid
2	16.11	Pentadecanoic acid, methyl ester	Saturated	0.09	Pentadecylic acid
3	18.013	Hexadecanoic acid, methyl ester	Saturated	15.49	Palmitic acid
4	18.453	9-Hexadecenoic acid, methyl ester, (Z)-	MUFA	1.44	Palmitoleic acid
5	19.773	Heptadecanoic acid, methyl ester	Saturated	0.88	Margaric acid
6	20.174	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	Saturated	0.87	
7	20.358	Tetracosanoic acid, methyl ester	Saturated	1.84	Lignoceric acid
8	21.937	Cyclopentanetridecanoic acid, methyl ester	Saturated	16.56	
9	22.414	9-Octadecenoic acid (Z)-, methyl ester	MUFA	17.84	Oleic acid
10	22.851	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	PUFA	17.4	Leinoleic acid
11	23.666	10-Nonadecenoic acid, methyl ester $$	MUFA	0.37	
12	23.896	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	PUFA	0.63	α linolenic acid
13	24.273	6,9-Octadecadienoic acid, methyl ester	PUFA	1.87	
14	25.09	Eicosanoic acid, methyl ester	Saturated	7.03	Arachidic acid
15	25.412	11-Eicosenoic acid, methyl ester	MUFA	6.8	Gondoic acid
16	28.547	Docosanoic acid, methyl ester	Saturated	9.67	Behenic acid
17	28.917	13-Docosenoic acid, methyl ester, (Z)-	MUFA	0.95	Brassidic acid

Table 5: Fatty acids profiling of fried groundnut oil in fifth consecutive stage

Peak No.	Retention time	Name of the compound	Type of Fatty Acid	Area %	Compound name
1	5.77	Octanoic acid, methyl ester	Saturated	0.23	Caprylic acid
2	14.285	Methyl tetradecanoate	Saturated	0.23	Myristic acid
3	15.804	Dodecane, 1,1-dimethoxy-	Saturated	0.09	Lauric acid
4	17.998	Hexadecanoic acid, methyl ester	Saturated	13.91	Palmitic acid
5	18.324	7-Hexadecenoic acid, methyl ester, (Z)-	MUFA	0.5	
6	18.435	9-Hexadecenoic acid, methyl ester, (Z)-	MUFA	0.79	Palmitic acid
7	19.76	Heptadecanoic acid, methyl ester	Saturated	0.78	Margaric acid
8	20.339	Tetracosanoic acid, methyl ester	Saturated	1.82	
9	21.913	Octadecanoic acid, methyl ester	Saturated	17.57	Stearic acid
10	22.033	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	PUFA (trans)	11.04	
11	22.36	8-Octadecenoic acid, methyl ester	MUFA	8.88	
12	22.444	10-Octadecenoic acid, methyl ester	MUFA	0.78	
13	22.823	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	PUFA	14.59	Linoleic acid
14	23	9,12-Octadecadienoic acid, methyl ester	PUFA	2.42	Linoleic acid
15	23.175	9-Octadecenoic acid (Z)-, methyl ester	MUFA	0.23	Oleic acid
16	23.338	14-Pentadecynoic acid, methyl ester	MUFA	0.17	
17	23.64	9-Hexadecenoic acid, methyl ester, (Z)-	MUFA	0.32	Palmitic acid
18	23.869	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	PUFA	0.55	Alpha linolenic acid
19	24.25	8,11-Octadecadienoic acid, methyl ester	PUFA	0.91	
20	24.441	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	PUFA	0.52	Linoleic acid
21	25.065	Eicosanoic acid, methyl ester	Saturated	6.26	Arachidic acid
22	25.39	11-Eicosenoic acid, methyl ester	MUFA	6.51	Gondoic acid
23	25.748	Octadecanoic acid, 9,10,12-trimethoxy-, methyl ester	Saturated	0.4	
24	26	Cyclooctanecarboxylic acid, 4,5-dimethyl-, methyl ester	Saturated	0.21	
25	26.133	9,12-Octadecadienoic acid, methyl ester	PUFA	0.25	Leinoleic acid
26	26.643	Heneicosanoic acid, methyl ester	Saturated	0.2	
27	28.532	Docosanoic acid, methyl ester	Saturated	8.5	Behenic acid
28	28.897	13-Docosenoic acid, methyl ester, (Z)-	MUFA	0.79	Erucic acid
29	30.025	Octadecanoic acid, 9-oxo-, methyl ester	Saturated	0.23	Caprylic acid
30	30.568	Tetracosanoic acid, methyl ester	Saturated	0.32	Myristic acid

Table 6: Fatty acid composition of frying oil after Third, Fourth and Fifth time

Type of Fatty Acid	Third Stage (oil)	Fourth Stage (oil)	Fifth stage (oil)
Saturated	41.59	52.7	50.75
Unsaturated	58.41	47.3	49.25
MUFA	39.2	27.4	18.97
PUFA	19.21	19.9	19.24
Trans	0	0	11.04
MUFA+PUFA/SFA	1.404	0.898	0.753
PUFA/SFA	0.462	0.378	0.379
MUFA/SFA	0.943	0.520	0.374

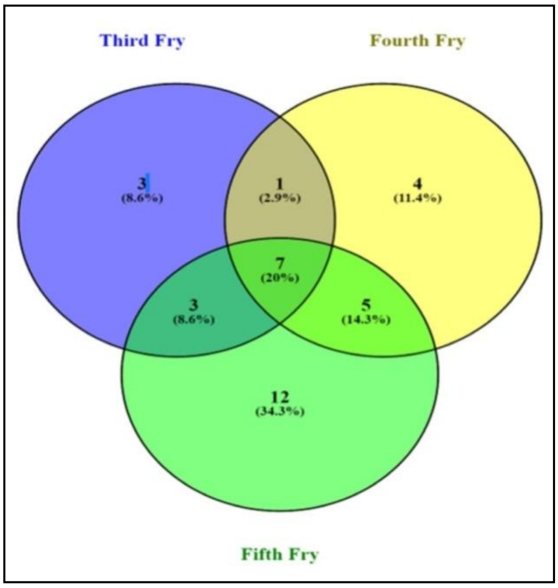

Fig 1: Ven diagramme of common fatty acids in frying process.

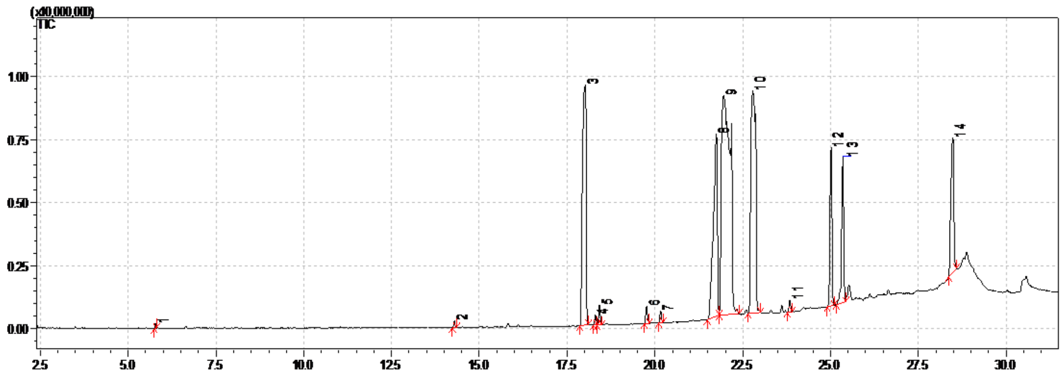

Fig 2: Fatty acids profiling of groundnut oil after third frying stage

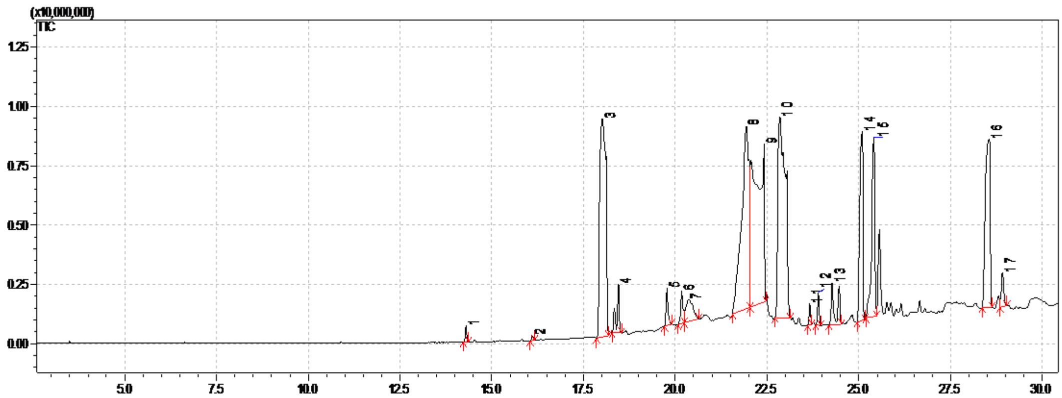

Fig 3: Fatty acids profiling of groundnut oil after fourth frying stage

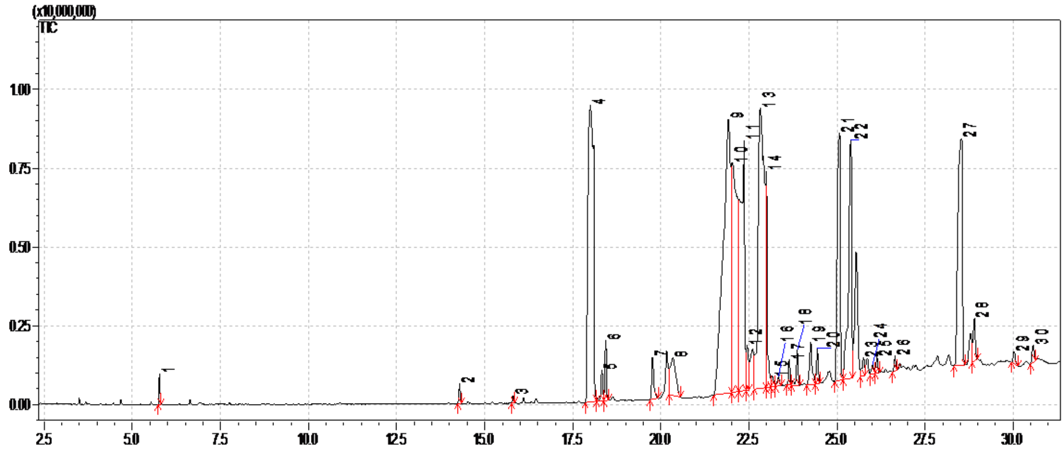

Fig 4: Fatty acids profiling of groundnut oil after fifth frying stage

Conclusions

Shelf life of peanut oil is about six months in ordinary conditions. And its quality may remain good for up to nine months in packed condition but repeated use of cooking oil convert in trans, the oil is especially rich in mono-unsaturated fatty acids (MUFA) like oleic acid that helps to lower LDL or bad cholesterol and increases HDL or good cholesterol in the blood. Research studies suggest that diet rich with monounsaturated fatty acids help to prevent coronary artery disease (CAD) and strokes by favoring healthy blood lipid profile. But our results suggested that up to third stage repetition of oil for frying is advisable to use but after that it showed 1 to 11% production in trans fat, therefore it is not advisable to reuse of refrying oil for food preparation.

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