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Investigation of physico-chemical properties of edible oils during frying with special reference to *in vitro* lipid peroxidation as TBARS

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Abstract
Reusing cooking oil in food preparation, especially during deep-frying, is a common practice to save costs. Repeated heating of the oil accelerates oxidative degradation of lipids, forming hazardous reactive oxygen species and depleting the natural antioxidant contents of the cooking oil. In the present study, popular edible oils, such as, mustard oil, soya bean oil and sunflower oil were subjected to frying till 8hr and samples were taken at 0hr, 2hr, 4hr, 6hr and 8hr and subjected to physico-chemical analyses including Malondialdehyde (MDA) expressed as Thiobarbituric Acid Reactive Substances (TBARS).It was observed that there were significant increases in free fatty acid (FFA), peroxide value, p-anisidine value,. Total Oxidation Index (TOTOX value) and total polar compounds whereas significant decrease was observed in iodine values with increase in frying time. Fatty acid profiling revealed increase in SAFA and MUFA and significant decrease in PUFA. The results indicated that the amount of malondialdehyde formation as TBARS was increased in the all of the edible oil samples due to repeated frying (from 32.14 µM at 0 hr to 40.02 µM at 8 hr for soya bean oil; from 24.51 µM at 0 hr to 35.71 µM at 8 hr for sunflower oil; from 12.14 µM at 0 hr to 21.22 µM at 8 hr for mustard oil;). The findings suggest high degree of thermal oxidation in edible oil samples due to repeated frying process.

Keywords: Edible oil, frying, MDA as TBARS, oxidation

1. Introduction
Deep frying is the most common and one of the oldest methods of food preparation worldwide. It involves heat and mass transfer. To reduce the expenses, the oils tend to be used repeatedly for frying. When heated repeatedly, changes in physical appearance of the oil will occur such as increased viscosity and darkening in colour, which may alter the fatty acid composition of the oil. Heating causes the oil to undergo a series of chemical reactions like oxidation, hydrolysis and polymerization [1, 2]. During this process, many oxidative products such as hydroperoxide and aldehydes are produced, which can be absorbed into the fried food [3]. Thermally oxidized oils such as those produced by repeated frying, contain a complex mixture of products, such as oxidized monomers, dimmers and polymers [4]. These products have been reported to be the substances mainly responsible for changes in the physicochemical properties of fats [5]. Free radicals generated during repeated frying process could damage lipids by initiating lipid peroxidation [6]. Malondialdehyde, one of the major secondary oxidation end products of peroxidised PUFA, has been shown to be of biological significance [7].

2. Materials and Methods
Edible oils were purchased from local market of Kolkata, West Bengal, India. Non-stick pan was used for carrying out the frying operations. Potatoes were purchased from the local market.
All the reagents and chemicals used were of analytical grade and procured from E-Merck India Ltd.

2.1 Analysis of the fatty acid composition of extracted lipid by Gas-Liquid Chromatography
The fatty acid composition of the edible oil was determined using Gas-Liquid Chromatography according to the AOCS 7th edition 2017, Official method Ce 1-07 using DB-Wax capillary column (30mX0.32mmX0.25µm) and a flame ionization detector [11].

2.2 Frying of potato chips

The locally purchased potato was peeled, washed by water, and sliced of equal dimension (0.5 cm) (200 g) were fried 1L of each oil samples separately at 180-190 °C. Oil samples were pooled once at the beginning (0 hr), second after 2 hr, third after 4 hr, fourth after 6hr and at the end of 8 hr. The temperature maintained during the whole experiment, including the frying operation, was within the range of 180 -190 °C. The heating of the oil was carried out in a non-stick frying pan to prevent the leaching of metals from utensils. This is also to mention that neither salt nor any other spices were added during the frying of the potato chips. The process was depicted in mentioned flow chart.

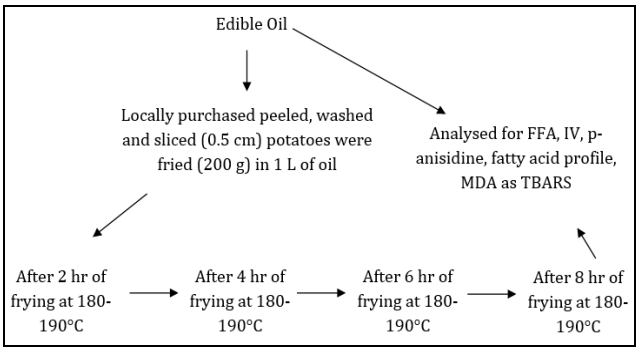

Fig: Oil samples

2.3 Oil samples

Oil samples were collected every 2hr and kept in refrigeration. Fresh oil was never added to the frying vessel for replenishment.

2.4 Oil analysis

The oil quality parameters *viz*. free fatty acid, iodine value, peroxide value, p-anisidine value and fatty acids were determined for fresh oil as well as for the heated oil samples collected every 2 hr of heating.

For determination of acid value, iodine value, peroxide value, and p-anisidine value, FSSAI Lab Manual for Oils and Fats methods were used. Total polar compounds were estimated according to AOAC method. Total Oxidation Index (TOTOX) was calculated as follows [9]:

TOTOX = p-anisidine value + 2 peroxide value

2.5 Measurement of MDA in the samples

The level of MDA in the samples was determined before and after frying. One gram of each oil sample was completely dissolved in a test tube containing 5 ml of acetic acid. The mixture was shaken for 1 hr and filtered using a filter paper. The filtrates were centrifuged for 10min and the supernatant was used for the analysis. The supernatant (1 ml) was mixed in a tube containing TBA (1 ml).The tubes were placed in a water bath for 1 hr at 95 °C. They were cooled at room temperature. The absorbance of mixtures was measured by spectrophotometer in three replicates. The level of TBARS (μM MDA/g) was calculated in the samples using the standard curve [10].

3. Results and Discussion

3.1 Physico chemical analysis: Estimation of edible oil samples (0 hr, 2hr, 4hr, 6hr and 8hr) were carried out for free fatty acid, peroxide value, iodine value, p-anisidine, TOTOX and total polar compounds. It was observed that values of free fatty acid, peroxide value, p-anisidine, TOTOX and total polar compounds increased significantly with increasing frying time whereas iodine value was found to decrease. These results signify progression of thermal oxidation and polymerisation of oil with increasing frying time. The results were depicted in Tables 1-2.

3.2 Fatty acid Profiling

Fatty acid profiling of edible oil samples (0 hr, 2hr, 4hr, 6hr and 8hr) were carried out and it was found that saturated fatty acid (SAFA) and mono unsaturated fatty acid (MUFA) increased and poly unsaturated fatty acids (PUFA) decreased with frying time. This signifies thermal breakdown of unsaturated double bonds. The results were depicted in Table 3.

3.3 Lipid Peroxidation as TBARS

The calibration curve was prepared using different concentrations of MDA in the range of 10 to 160 μM. The stock solution of MDA gave an accurate standard curve with high repeatability in the spectrophotometer. Linear regression in the calibration curve of MDA showed a correlation coefficient of 0.999 (Figure 1).

The TBARS values in the edible oil samples were found to increase with frying time. The results were incorporated in Table 4, Figure 2.

Table 1: Analysis of edible oils after total 8 hr of frying

Sample	Acid Value					Peroxide Value					Iodine value				
	0 hr	2 hr	4 hr	6 hr	8 hr	0 hr	2 hr	4 hr	6 hr	8 hr	0 hr	2 hr	4 hr	6 hr	8 hr
Soyabean Oil	0.12	0.15	0.17	0.35	0.47	0.24	0.37	0.49	0.81	0.99	126.46	122.97	115.47	96.83	90.24
Sunflower Oil	0.15	0.27	0.29	0.35	0.57	0.28	0.31	0.37	0.49	0.62	123.97	124.51	121.54	115.39	107.85
Mustard Oil	0.66	0.74	0.92	1.24	1.45	0.41	0.67	0.72	1.21	2.32	107.54	107.14	96.14	90.21	87.62

Table 2: Analysis of edible oils after total 8 hr of frying

Sample	p-anisidine value					TOTOX					Total polar Compounds (%)				
	0 hr	2 hr	4 hr	6 hr	8 hr	0 hr	2 hr	4 hr	6 hr	8 hr	0 hr	2 hr	4 hr	6 hr	8 hr
Soyabean Oil	3.15	4.85	5.96	8.41	11.74	6.54	10.07	12.41	17.63	24.47	BDL(DL:0.5)	0.84	3.71	5.71	9.1
Sunflower Oil	2.04	2.34	3.89	6.71	9.07	4.36	4.99	8.15	13.91	18.76	BDL(DL:0.5)	0.67	1.74	3.71	4.08
Mustard Oil	0.71	2.85	5.71	6.14	8.04	1.83	6.37	12.14	13.49	18.4	BDL(DL:0.5)	3.62	7.41	10.41	11.96

Table 3: Analysis of Fatty Acids after total 8 hr of frying

Sample	Saturated Fatty Acid (%)					Mono-unsaturated Fatty Acid (%)					Poly-unsaturated Fatty Acid (%)				
	0 hr	2 hr	4 hr	6 hr	8 hr	0 hr	2 hr	4 hr	6 hr	8 hr	0 hr	2 hr	4 hr	6 hr	8 hr
Soyabean Oil	17.29	17.86	18.32	19.01	19.42	22.86	22.91	23.41	23.71	23.99	59.83	59.23	58.27	57.28	56.59
Sunflower Oil	10.39	10.41	11.42	11.71	13.52	19.41	19.56	20.52	20.67	21.05	70.2	70.03	68.06	67.62	65.43
Mustard Oil	6.09	6.72	7.21	7.45	7.96	64.72	65.41	66.01	66.85	66.96	29.2	27.87	26.78	25.7	25.08

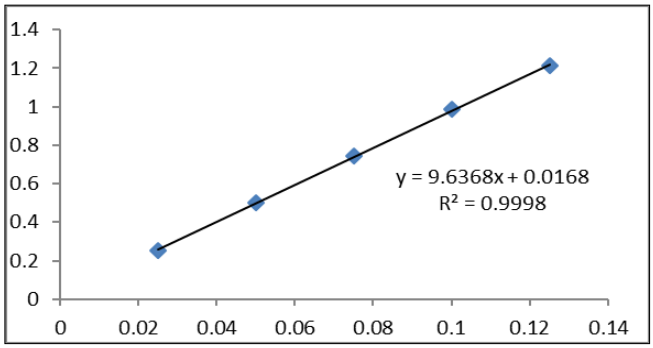

Fig 1: Standard Curve of MDA

Table 4: Estimation of MDA as TBARS in Edible oils

Sample	MDA as TBARS (uM)				
	0 hr	2 hr	4 hr	6 hr	8 hr
Soyabean Oil	32.14	33.41	36.52	38.71	40.02
Sunflower Oil	24.51	27.52	29.14	32.52	35.71
Mustard Oil	12.14	13.58	16.87	19.71	21.22

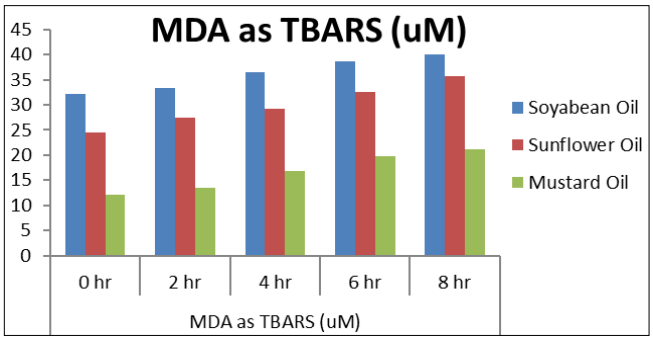

Fig 2: Estimation of MDA as TBARS in Edible oils

4. Conclusion

Deep-fat frying causes the hydrolysis, oxidation, and polymerization of the oil. Hydrolysis increases the amount of free fatty acids, mono- and diacylglycerols, and glycerols in oils. Oxidation occurs at a greater rate than hydrolysis during deep-fat frying. Oxidation produces hydroperoxides and then low molecular volatile compounds such as aldehydes, ketones, carboxylic acids, and short chain alkanes and alkenes. Dimers and polymers are also formed in oil by radical and Diels-Alder reactions during deep-fat frying.

Lipid peroxidation have been postulated to be the destructive process of liver. In the present study, elevations in the levels of lipid peroxidation as TBARS were observed. In conclusion, the use of edible oils after repeated deep frying for cooking should be avoided regardless of the economic disposition.

In the present study, oxidation levels increased in all products and there was a direct association between the food product and lipid oxidation. Therefore, the determination of MDA content in food can be used as a proper tool for monitoring the levels of lipid peroxidation and the safety of fried products.

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