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A STUDY OF TOTAL POLAR MATERIAL, ACID VALUE AND PHYSICAL PARAMETERS TO ASSESS LIPID OXIDATION STATUS

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ABSTRACT

Lipid oxidation on repeated heating alters its chemical composition due to generation of various by-products which also affects its physical properties. To determine the correlation of accumulated total polar materials and generated free fatty acids with physical parameters, quality degradation of an unbranded mustard oil were evaluated after subjecting the oil to repeated oxidation through deep-frying of snacks- *onion fritters* and *lentil fritters* (split black-lentil fritters), separately without replenishment of oil for seven successive times, at an interception of 48 hours. Thermal stability indices investigated were colour, refractive index, viscosity, smoke point, acid value, and total polar material. With subsequent frying operation, these values increased in both items recording maximum deterioration in *onion fritter*-oil with an accumulation of sulphur from onion. Constituent of food items along with moisture acted as a vector in frying instability of oil.

KEYWORDS

Lipid oxidation, Acid value, Total polar material, Viscosity, Sulphur accumulation and Smoke point.

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INTRODUCTON

Deep-fat frying is a complicated thermo-chemical process that affects the composition of both oil and the product being fried[1]. This undesirable change in the lipid composition is associated with formation of products like hydroperoxides[2,3] alkanes, alkenes (conjugated diene-triene)[3,4] aldehydes (both volatile and non-volatile), ketones, alcohol, esters, acids of low molecular weight and hydrocarbons[3]. The moisture, constituents present in foods, atmospheric oxygen, and high temperatures leads to reactions such as hydrolysis, oxidation and polymerization[2]. The chemical composition of the used frying oils modify due to release of free fatty acids and free radicals that in turn combine to produce monoglycerides, diglycerides and polymeric trigylcerides. All of these products are considered as polar compounds and grouped under the term total polar compounds, which is a reliable benchmark for measuring the degradation of the oil. Since these polar compounds are not digestible, consumption can impact consumer health, posing a greater risk of heart disease in the long term and gastrointestinal disorders in the short term.

Vegetable oil extracted from seeds of *Brassica nigra* (black mustard) is a popular cooking medium in Indian subcontinent[5]. The composition of the oil constitutes 80% unsaturated fatty acid comprising of 21% polyunsaturated fatty acid consisting with 6% ω-3 alpha-linolenic acid and 15% ω-6 linoleic acid[6] (in ratio 1:10). Since mustard oil has high smoke point it is popularly used as a deep- frying medium. While deep frying different snacks, food vendors in unorganized sector often reuse heat-abused oil in batch processes without replenishing with fresh oils. Deep frying requires presence of substantial amount of excess oil, which has been already subjected to high temperature and is leftover in the fryer at end of each frying. This surplus oil is often used for next day's frying along with fresh oil top-ups whenever required. Hence it can be a potential threat to the consumer public health owing to the frying medium abuse. Though researches suggest deep- frying in mustard oil reduce risks of heart attack[5] by almost 71% much study is not documented on the quality deterioration of mustard oil on repeated oxidation and its variation with varying food products.

In the present investigation, lipid deterioration due to oxidation is evaluated by subjecting the mustard oil to high temperature repeatedly while deep frying two popular evening snacks viz. onion fritter (gram-flour batter coated onion) and lentil fritters (gram flour batter of split black lentil) separately at a fixed interception of 48 hours. This was also done with an objective to determine the correlation of the chemical parameters *viz*. Total polar material and acid value and also impact of sulphur[7,8] present in onion with the physical parameters tested and determine the extent of oxidation /spoiling of oil. Two different food products were chosen to study the variation of chemical interaction of food with oil subjected to oxidation.

MATERIAL AND METHODOLOGY

Chemicals

Silica gel for chromatography, EDTA, EBT, barium chloride, magnesium chloride, potassium hydroxide and all other solvents and reagents were procured from Merck (India). All chemicals used were of analytical grade.

Oil

Non-branded mustard oil (3 kg) was procured from the local market of Kolkata, India.

Sample Preparation and Frying Operation

To prepare onion fritter 15 grams onion (*Allium cepa*) were peeled, thin sliced and mixed with 6 grams of gram-flour, 3 grams of salt, 3 grams of chili powder and 6 ml of water. The mixture was then shaped into three fritters.

To prepare lentil fritters 15 grams of powdered split black lentil (*Vigna mungo*) powder were mixed with 6 grams of salt, 3 grams of chili powder and 6 ml of water. The batter was prepared and shaped into three fritters.

For deep frying, two sets of 1 kg of mustard oil were heated to smoke point and respective items were fried in it for 2 minutes. This process was repeated 7 successive times for each item at an interception of 48 hours.

To stimulate a controlled frying and to study the deterioration of oil at frying temperature, without the food being fried another set of 1 kg oil (without any food sample) was heated to smoke point with addition of measured amount (6 ml) of water for 7 successive times.

After each frying, the samples were allowed to cool to room temperature and stored in glass bottles filled up to the brim ensuring no head space oxygen. The lid were sealed with paraffin wax and kept in dark and refrigerated to stop the diffusion of gas across as well as any further chemical reactions.

Various parameters associated with lipid deterioration of the frying oil before and after heat treatment were determined following standard procedures.

PHYSICAL TESTS

Smoke Point

Smoke point was recorded with a mercury thermometer before each frying.

Colour Index

Absorption of the oil samples at 420 nm was determined on a UV–Spectrophotometer (U–2000, Hitachi, Tokyo, Japan) and the values were taken against water as blank.

Viscosity

Viscosity was measured by using Ostwald's Viscometer.

Refractive Index

Refractive Index of the oil samples were measured using an Abbe's Refractrometer.

Sulphur Test

It was done only for the oil in which onion fritter was fried since onion contains considerable amounts of sulphurous compounds.

A volume of 25 ml of the sample was pipetted out, diluted to 50 ml with acetone. The pH was adjusted to 1 with 2 M HCl. The solution was heated to boil. To it was added 15 ml of boiling 0.05 M $BaCl_2$ solution. Subsequently 35 ml of 0.5 M standard EDTA solution and 5 ml of ammonia solution was added and boiled for 15-20 minutes and cooled. About 10 ml of buffer solution of pH 10 and a few drops of Eriochrome black T (EBT) indicator was added and titrated with 0.05 M $MgCl_2$ solution to a clear red colour. Weight percent of sulphur was calculated using the formula:

$$\text{Sulphur} = \frac{32 \times 0.0116 \times \text{Volume of } BaCl_2 \text{ and EDTA complex} \times 100}{233 \times \text{Weight of sulphur in grams}}$$

Acid Value

In a conical flask, 25 ml of diethyl ether was mixed with 25 ml alcohol and 1 ml of phenolphthalein solution. The mixture was carefully neutralized with 0.1 N sodium hydroxide. This is called Neutral Solvent. 2 grams of oil sample was dissolved in the neutral solvent and was titrated with aqueous 0.1 N sodium hydroxide, shaking vigorously until a pink colour was formed that persisted for 15 seconds. Acid value was calculated as:

Acid value = (Volume of sodium hydroxide solution (ml) x 5.61) / Weight of the oil sample.

Total Polar Material Estimation

It was done by column chromatography and distillation. The recovered substance was verified by thin layer chromatography.

Statistical Analysis

The experiment was performed in triplicate and data from three different experiments were subjected to analysis of variance (ANOVA) ($P< 0.05$). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 16.0 for windows, SPSS Inc.)

RESULTS AND DISCUSSION

Loss of Weight in the Items after Frying

After frying of fritters it was observed that though both of them was treated with same amount of water for making the batter, onion fritter suffered a greater loss in weight(45%) on frying than the lentil (20%) (Table No.1). In the deep frying process, water content of food creates a protective layer that simultaneously prevents penetration of oil in the food product and allows the product to cook thoroughly[9]. As the water evaporates, fatty acids, monoglycerides and diglycerides are released. During hydrolysis, water cleaves the ester bond of oil and releases glycerol and fatty acid. The hydrolysis derivatives increase the oxidation and reactivity higher than the original triacylglycerides, which in turn accelerate degradation. The intensity of this process enhances for food with higher water content which interacts with the frying oil or fat[10].

This loss of weight in case of onion fritter may be accounted for higher water content of onion (84.3%) than the split black lentil (10.9%).

Smoke Point

The smoke point recorded a steady significant fall ($P>0.05$) with subsequent heating (Figure No.1). The maximum value noted for onion fritter-oil indicated a direct dependence of the smoking point not only on the number of frying but also on the constituents of the food items selected.

Colour Index

Darkening of oil colour during deep fat frying is mostly due to unsaturated carbonyl compounds and nonpolar compounds. Maillard browning products are also the major contributors of discoloration of the oil. Colour changes are also related to the formation of hydro-peroxides, aldehydes and ketones[11]. Additional cause of colour changes are the presence of pigments present in the oil[12] as well as which is leaching out from the food during frying. Hence colour index reflects over the degradation of oil. Here the colour intensity increased uniformly (P>0.05) with successive frying. In control, from the first frying to the seventh frying colour index increased by 3.28%, whereas the intensification observed in onion fritter and lentil fritters was respectively 7.34% and 3.8% between the initial and final frying operation. A maximum rise was observed in between second and fourth frying in onion fritter and between first and third frying in lentil fritters fried oil. The colour index value recorded was highest in control, though the increase in the colour index range was highest in onion fritter (Figure No.2). Presence of sulphurous compounds in onion, which was getting transferred in the frying medium, might contribute to higher gradient in colour index values of onion fritter oil. There is a moderate positive correlation of 0.56 between the colour index and sulphur (Table No.2). Acid value graph show a similar order as colour index, recording highest set of values for control, followed by lentil fritter and onion fritter oil respectively. Acid value also has a high positive correlation with colour index (Table No.2).

Viscosity

Increase in the viscosity (P>0.05) of the frying oil was found to be directly proportional to the number of frying operation. Maximum viscosity increase of 494% between the initial and final frying was observed in case of onion fritter, with a sudden leap in value between first and second frying. Similar increases recorded for lentil fritters and control were 306% and 370% respectively. An abrupt change in the value occurred between sixth and seventh frying in lentil fritters whereas in control it happened between first and second frying (Table No.1). Viscosity of frying oil increases as a consequence of formation of high molecular weight polymers[12]. Combination of free radicals with fatty acid leads to formation of both linear and cyclic products resulting in dimers, trimers and polymers of the triacylglycerides. These polymers enhance the viscosity of the oil and also lead to foam formation[13].

Oxidation of frying oils is also responsible for increasing viscosity due to accumulation of oxidized lipids[12]. Even the accumulation of polar compounds is found to attribute to increasing viscosity[14]. The interaction of moisture with the components of the oil, brings deteriorative changes which leads to formation of higher viscous substances. These points are corroborated from the high positive correlation values of total polar material and viscosity (Table No.2). All these factors collectively might have played a role in raising the viscosity of the oil.

Refractive Index

Refractive Index in both food items illustrated a gradual increase (P>0.05) in the value with successive frying. It recorded 29.65% increase in onion fritter, 30.77% in lentil fritters and only by 5.1% between first to seventh frying in control (Figure No.1). An abrupt change occurred in onion fritter between first and third frying whereas control showed a gradual increase in refractive index. Change in the refractive index was because of increased density of the oil due to more number of frying. Higher refractive index value for control compared to onion fritter and lentil fritter oil might be partially due to the presence of moisture whereas the increase in values in the other two may be due to the presence of sulphurous compounds and the incomplete amino acids present in the onion and in the split black lentil respectively.

Sulphur Accumulation

Significant accumulation of sulphur (P>0.05) was observed in the frying medium for onion fritter which increased with subsequent frying (Figure No.1). The presence of sulphur in the oil might play a role in increasing the colour index of the frying oil (moderate positive correlation 0.56) as indicated in Table No.2.

Total Polar Material

During deep-fat frying there is a formation of polar materials like aldehydes, ketones, acids etc. which varies with time, temperature and various other factors. Total polar material is a more reliable indicator of the quality of frying oil than the free fatty acid value since it accounts for all of the degradation products due to oxidation, polymerization and hydrolysis of oil during the deep frying process. Total Polar Compounds thus formed in successive frying increases the loss of water from food product and enhances fat absorption in the food product[15]. A frying life is considered to come to an end if the TPM value reaches 24 %[16]. In the two samples and control the TPM showed a proportional rise in value with subsequent frying process (P>0.05). The highest accumulation of polar material was recorded in onion fritter oil (90%) whereas the other two were comparatively less exhibiting a maximum of 75% and 56% in lentil fritters and control (Figure No.5). The TPM values thus established the non-usability of the oil right after the first frying.

Acid Value

The acid value measures the amount of carboxylic acid groups in free fatty acids generated in the oil after frying. It could also be used as an indicator of lipid oxidation and reusability of oil since increase in this value leads to formation of off-flavour as a result of degradation of oil[17,18]. The acid values increased significantly (P>0.05) with each frying. In onion fritter fried mustard oil the acid value showed a total increase of 84.38% with a sudden change of 58.27% between the 1st and 2nd frying. In case of lentil fritters and control acid value increased by 82.63% and 81.25% respectively (FigureNo.2). Presence of moisture during heating or cooling the oil (<200° F) increases the acid value. Development of free fatty acids in oil leads to depression of the smoke point of the heated oil, and contribute to undesirable smells and flavors[19]. This has perfectly corroborated with the high negative correlation of acid value and the smoke point (Table No.2).

Statistical Analysis

The overall view of the results of the ANOVA indicted that the variation in the food item had significant impact on oxidative parameters. Thus it can be inferred that the kind of food sample had a control on the oxidation of oil. The lipid oxidation responded to the variation in frying time as well as the assorted food items used for frying.

Correlation Study

Correlation of various evaluated physical parameters of lipid oxidation and sulphur accumulation with the chemical parameters viz. acid value and total polar material was determined. Table No.2 represents the correlation values of the above mentioned parameters for the three different samples. The acid values and the total polar material of all the oxidized oil samples exhibited very high positive correlation with parameters like colour index, viscosity and refractive index. Since the smoke point decreased with frying, the acid value and the total polar material recorded a very high negative correlation with the smoke point of each type of oil. These indicate that with the increase of polar material increased the colour index, refractive index and viscosity. Sulphur accumulation was found to moderately correlate with colour index (0.56) whereas it negatively correlated with smoke point. These correlation values emphasize that more is sulphur, higher is the colour index and greater is the depression in the smoke point.

Table No.1: Variation in weight before and after frying of deep fried food varieties

S.No	Food items	Initial weight (gms)	Final weight (gms)	Water added (ml)	Reduction in weight (gms)
1	Onion fritter	11	6	2	5
2	lentil fritters	10	8	2	2

Table No.2: Correlation values between different physical and chemical oxidative parameters

CORRELATION VALUES				
acid value:				
	colour	**smoke point**	**Refractive Index**	**Viscosity**
Onion fritters	0.992246	-0.95472	0.918927	0.967726
Lentil fritters	0.892284	-0.98114	0.825202	0.983342
Control	0.958484	-0.92172	0.929944	0.988257
total polar material:				
	colour	smoke point	Refractive Index	Viscosity
Onion fritters	0.962048	-0.88147	0.95103	0.988862
Lentil fritters	0.959548	-0.89357	0.981554	0.858852
Control	0.965758	-0.91131	0.893537	0.993791
sulphur content:				
	colour	smoke point	Refractive Index	Viscosity
Onion fritters	0.55785	-0.70047	0.379933	0.368922
total polar material:acid value				
Onion fritters	0.976735			
Lentil fritters	0.848938			
Control	0.991001			

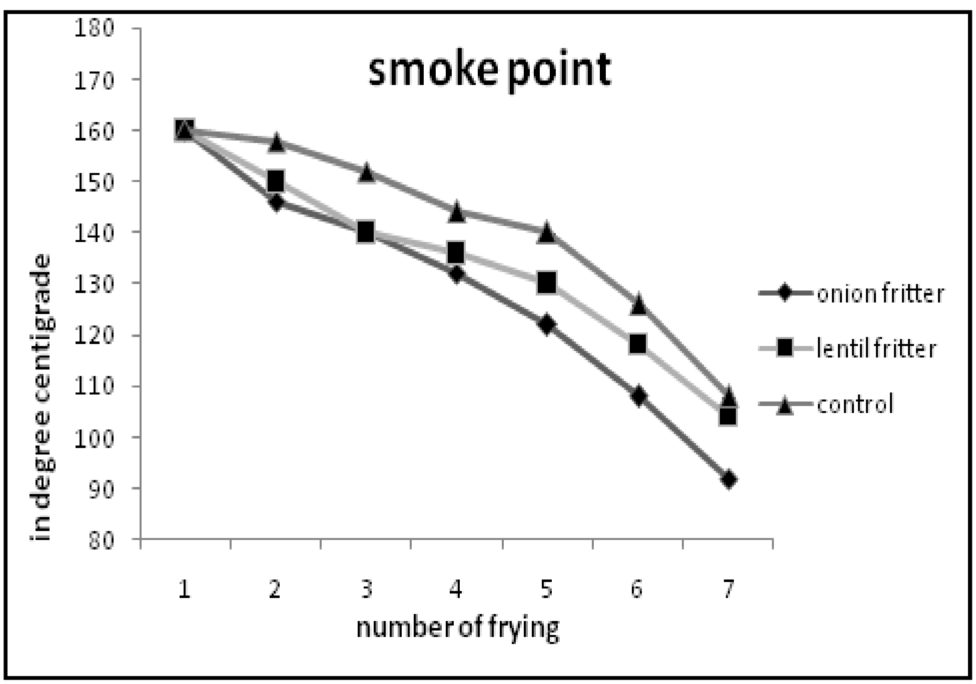

Figure No.1: Graphical representation of variation of smoke point of three oxidized oil samples on successive frying

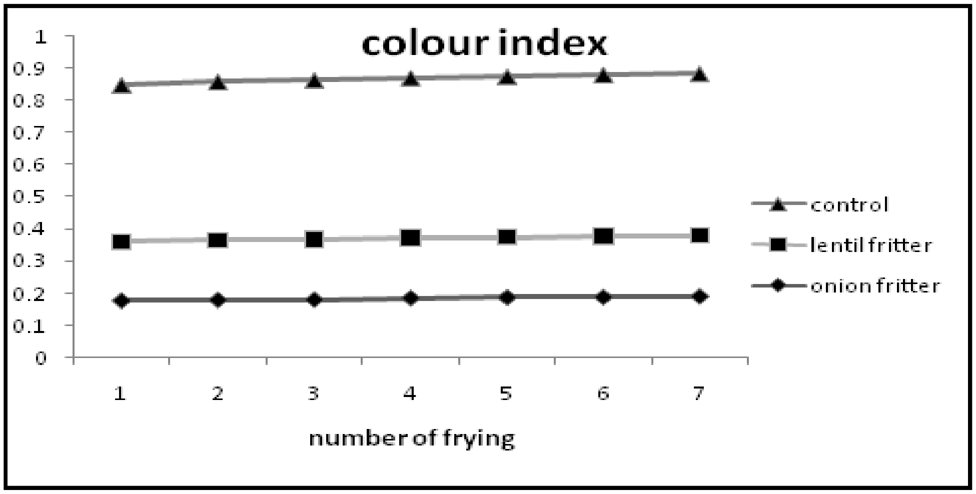

Figure No.2: Graphical representation of variation of colour index of three oxidized oil samples on successive frying

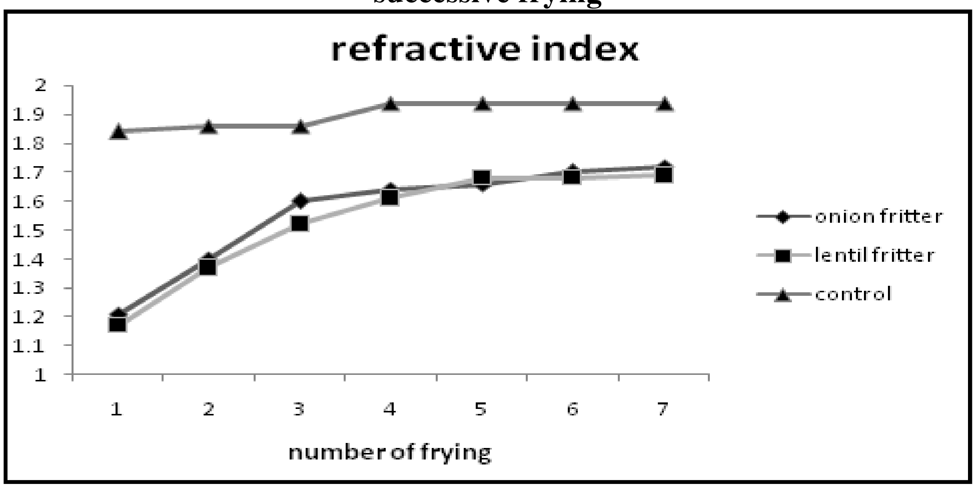

Figure No.3: Graphical representation of variation of refractive index of three oxidized oil samples on successive frying

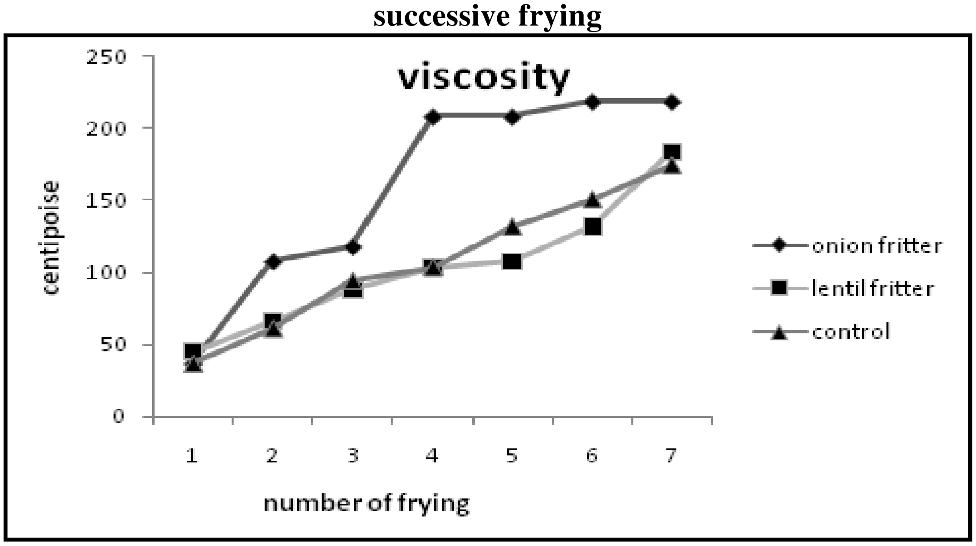

Figure No.4: Graphical representation of variation of viscosity of three oxidized oil samples on successive frying

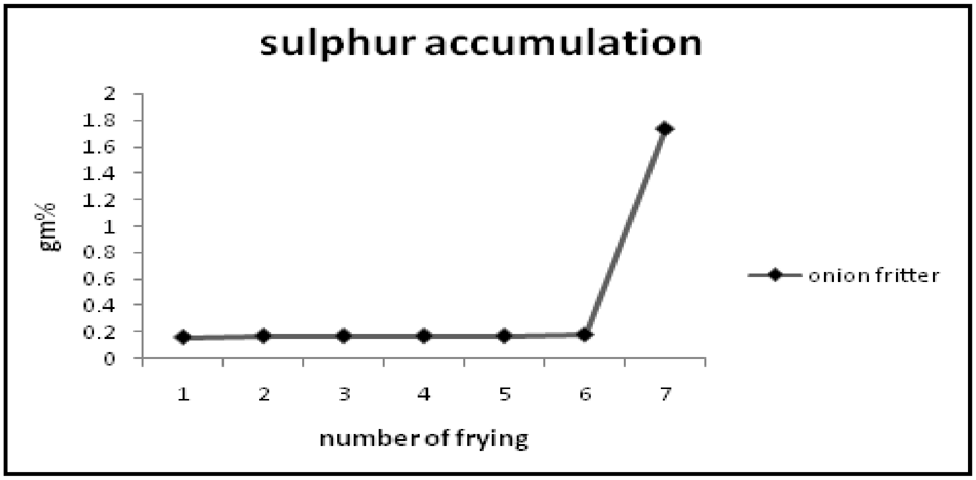

Figure No.5: Graphical representation of variation of sulphur accumulation of oil sample in which onion fritter is fried

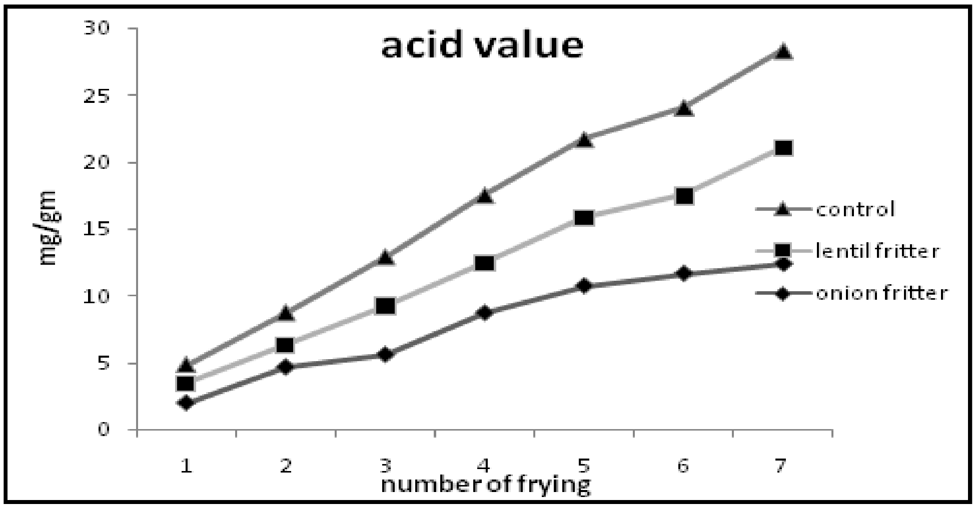

Figure No.6: Graphical representation of variation of acid value of three oxidized oil samples on successive frying

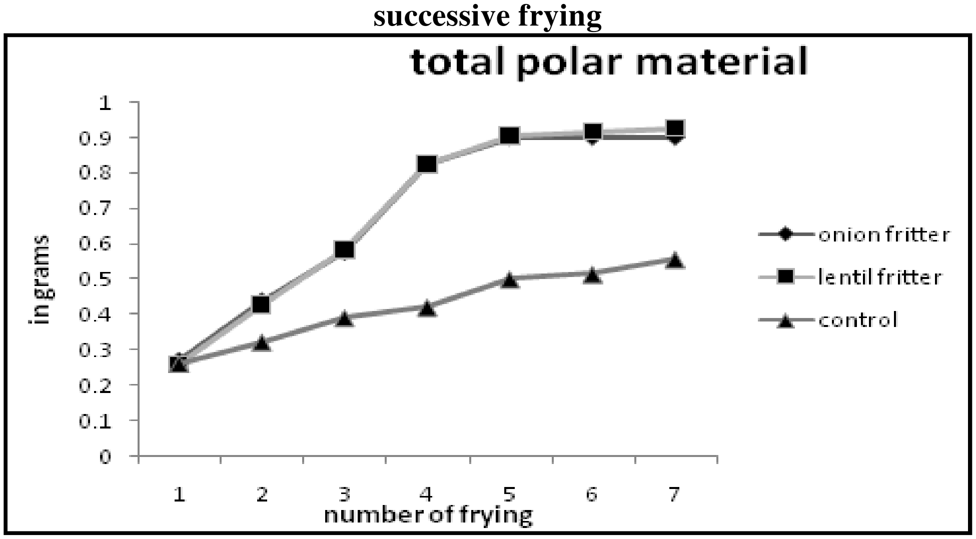

Figure No.7: Graphical representation of variation of total polar material of three oxidized oil samples on successive frying

CONCLUSION

In nutshell, it can be stated that lipid oxidation increases with successive times of heating. It is also dependent on the kind of item fried. Maximum oxidation as well as hydrolysis with high accumulation of different polar material and high acid value was noted in the oil in which onion fritters were fried compared to lentil fritter oil. All the physical parameters except the smoke point increased with each time heating and the physical parameters strongly correlated with the oxidative chemical parameters. Sulphur accumulation due to leaching occurred in case of onion species which enhanced the oxidation as well as hydrolysis as observed from the acid value increase which is also due to the high water content of onion. The degradation of oil has occurred in all the cases to such an extent as evaluated from the above stated physico-chemical parameters that the oil became unusable after the first operation itself.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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