

OXIDATIVE STABILITY OF CANOLA OIL BY PHYSICO-CHEMICAL ANALYSIS AND FT-IR SPECTROSCOPY

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ABSTRACT

This study was conducted to investigate the stability of canola oil after convention heating and repeated frying with potato piece. The progress of lipid oxidation was assessed in terms of physicochemical properties like viscosity, density, saponification value, peroxide value and acid value. Percentage free fatty acids (FFA) and percentage of glycerol present in each type of triglycerides was also calculated. Oxidative stability of the oil was studied by Fourier Transform-Infrared (FT-IR) spectroscopy. Results revealed that proportions of fatty acid changes with heating and frying, in result changes were observed in the intensities of spectral bands. Formation of secondary oxidation products were showed by the peak at frequency range $3753.48-3750\text{ cm}^{-1}$. Degree of chain length and degree of branching of fatty acids were calculated by FT-IR data.

Keywords: canola oil, physicochemical characteristics, convention heating-frying, carbohydrate, FT-IR analysis

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INTRODUCTION

Lipids are the main constituent of edible oil, which are triacylglycerol (TAG) molecule. TAG is an ester of glycerol containing three long chain fatty acids. These fatty acids are present in saturated, mono-unsaturated and poly-unsaturated forms. Palmitic acid and stearic acid are saturated fatty acids whereas oleic acid and linolenic acid are unsaturated ingredients. Oils are rich source of energy and nutrients which significantly contribute for the development and regulation of different functions of human body. Edible oils are mostly used in our daily life for cooking, pan-frying, baking and deep-frying of different food items to get required flavor, color and crispy texture¹. The unsaturated ingredient of the oil affects the nutritive value of oil and is prone to oxidation, isomerism, hydrolysis, cyclization and polymerization and cause negative impact on human health¹⁻². They contain fatty acids, antioxidants, antifoaming, anti-surfactant and less amount

of meal which are pivotal in preventing cardiovascular diseases, lung and breast cancer, inflammatory diseases and many other diseases by the intake of oil of foods such as soybean, olive, mustard, corn and canola etc³⁻⁴.

In canola oil, the total lipids contain 94.4-99.1 % of triacylglycerol. It is widely used as cooking oil due to high proportion of mono-unsaturated fatty acid (C_{18:1 n9} oleic acid ~60.0%) and low proportion of poly-unsaturated fatty acid. It requires little hydrogenation to reduce linolenic and linoleic acid to attain good frying stability. Stability of oil is affected by light oxygen partial pressure, excess of hydrogen, degree of unsaturation of fatty acids, presence of light, heat, anti-oxidants and pro-oxidants such as copper, iron and pigments.

Physicochemical parameters of oil are greatly influenced by kind and proportions of fatty acids. Heating and frying create chemical and physical changes in the composition of oil which can be determined by various tests such as viscosity, specific gravity, boiling point, saponification

value, acid value, iodine value and peroxide value. Presence of tocopherols, sulfur and linolenic acid in canola oil acts as anti-oxidants and improve the stability of oil on heating and frying. By the application of heat, loss of tocopherol occurs first due to its low rate of evaporation and destruction. Sulfur components form stable compounds by complexing hydroperoxy radicals and prevent from auto-oxidation. In triacylglycerol, the larger percentage of linolenic acid content is in sn-2 position which creates resistance of linolenic acid to oxidation.

Quantitative determination of oil samples was easily made by Fourier Transform-Infrared (FT-IR) spectroscopy which is simple, time and cost saving technique. For the analysis, low amount of sample is used and automatic operation reduces the chances of error. This technique gives the information about the oxidative changes of oil by the identification of different functional groups⁵. It is used to study the saturation and unsaturation composition of heated and unheated oils at room temperature for monitoring the oxidation process in oils.

This work aimed at investigating the oxidative stability of canola oil by studying physicochemical parameters of fresh oil and after storage. Effect of convention heating and repeated frying with a piece of carbohydrate were also studied to make understanding of the oil quality, stability and applicability after storage. The oxidation stability of oil was also studied by FT-IR spectroscopy.

METHODOLOGY

Chemicals and Reagents

Acetic acid (CH₃COOH), chloroform (CHCl₃), potassium iodide (KI), sodium thiosulphate (Na₂S₂O₃), potassium hydroxide (KOH), hydrochloric acid (HCl), 2-propanol were of A.R grade and purchased from Merck Darmstadt Germany. Canola oil was extracted from their dried, dehulled seeds purchased from local market in Karachi, Pakistan.

Convention heating process

Effect of convention heating on oil was determined by heating the oil at different temperatures. Samples were collected between 30°C to 60°C with interval of 10°C and at boiling point.

Frying process

Potatoes were peeled and cut into pieces (~5cm³) and continuously fried in same frying oil (50mL) five times. Duration of each frying was 10 minutes. After each frying sample was collected in vial and cooled to room temperature and stored for physicochemical analysis.

Density and viscosity measurement

Density of oil samples were measured by R.D bottle with a capacity of 10mL. The viscosity of fresh canola oil was measured by Ostwald Viscometer technicomail constant 0.05 Cs/c, ASTMAD 445 England. Stop watch (Japan, CBM, and Corp QSQ) with least count± 0.01s was used to record the flow time of oil⁶.

Saponification value

The saponification value was determined by accurately weighing oil sample and treated with 1N Alcoholic KOH and distilled water. Resultant mixture was heated under a reserved condenser to ensure that the sample was dissolved completely. Two drops of phenolphthalein were added and titrated with standard HCl until a pink color was remain for 30 seconds. A blank was determined with same time conditions without oil sample⁷.

$$SV = (B-S) \times N \times 56.1 / g \quad (1)$$

Where B is the mL of HCl used for blank, S is the volume of HCl used for sample flask, N is the normality of HCl, g is an accurate mass of the oil sample and 56.1g/mol is the molecular weight of KOH.

Acid value

For the determination of acid value known and accurate weight of oil sample was treated with neutralized 2-propanol and heated for 10 minutes. Phenolphthalein was added and titrated against standard KOH until pink color remains 30 seconds¹.

$$AV = \text{mL KOH} \times N \times 56.1 / g \quad (2)$$

Where N is the normality of potassium hydroxide solution, g is the amount of oil sample and 56.1g/mol is the molecular weight of KOH.

Peroxide value

Peroxide value is a measure of peroxides contained in the oil and was determined by measuring iodine released from potassium iodide. A known and accurate weight of oil sample was dissolved in acetic acid-chloroform (3:2) solution. Saturated KI solution and distilled water were added and shake the flask vigorously to liberate iodine from chloroform layer. The amount of iodine liberated from KI by oxidative action of peroxides present in the oil was determined by titration with standard sodium thiosulphate using starch solution as an indicator. Blank determination was also made¹.

$$PV = (S-B) \times N \times 1000 / g \quad (3)$$

Where B is the volume of sodium thiosulphate used for blank, S is the volume of sodium thiosulphate consumed by the sample flask, N is the normality of standard sodium thiosulphate and g is the amount of oil sample in gram.

FT-IR analysis

FT-IR spectra of canola oil samples before and after convention heating and repeated frying were recorded with the help of Fourier Transform Spectroscopy Model I-R Prestige 21 Shimadzu. A drop of each sample was poured between two disks of KBr and intensities were recorded in term of percentage transmittance (%T) between the ranges from 4000 to 400 cm⁻¹.

RESULTS AND DISCUSSION

The evidence of rancidity in canola oil was analyzed by physicochemical parameters such as density, viscosity, boiling point, saponification value, acid value and peroxide value. Table 1 show the initial quality of oil and

effect of storage on oil. The influence of convention heating and repeated frying with potato piece on canola oil after storage were shown in Table 2 and 3

respectively. Physicochemical properties of fresh canola oil are comparable to the values reported by Shazia et al., (2012).

Table 1. Physicochemical properties of canola oil at room temperature (30°C)

Properties	Fresh oil	After long storage	Reported by Shazia et. al., (2012)
Density (g/mL)	0.9075	0.9694	0.916-0.917
Viscosity (mPa s)	45.4	46	83.3
Saponification Value (mg KOH / g oil)	182	125.25	193.55
Peroxide Value (meq O ₂ / Kg oil)	21.52	152.066	3.05
Acid Value (mg KOH / g oil)	0.8059	9.423	1.12
% Free Fatty Acid	0.4054	4.7398	0.87
Ester Value (mg KOH/ g oil)	161.1941	48.147	192.43
% Glycerol	8.8173	2.6319	-

Table 2. Physicochemical properties of canola oil after convention heating

Properties	40°C	50°C	60°C	At boiling point
Saponification Value (mg KOH/g oil)	118.28	106.03	90.90	59.03
Peroxide Value (meqO ₂ /Kg oil)	240.48	220.31	197.39	175.68
Acid Value (mg KOH/g oil)	7.658	7.310	6.7071	6.308
% Free Fatty Acid	3.8519	3.6769	3.3737	3.1729
Ester Value (mg KOH/g oil)	110.622	98.72	84.1929	52.722
% Glycerol	6.0470	5.3964	4.6023	2.8819

Table 3. Physicochemical properties of repeated frying oil with carbohydrate five times

Properties	Frying Trials				
	1 st frying	2 nd frying	3 rd frying	4 th frying	5 th frying
Saponification Value (mg KOH/g oil)	5.031	9.960	16.38	23.67	42.795
Peroxide Value (meqO ₂ /Kg oil)	107.39	16.271	10.56	8.517	4.425
Acid Value (mg KOH/g oil)	7.4761	6.7955	6.5393	4.9084	4.559
% Free Fatty Acid	3.7604	3.4181	3.28926	2.4689	2.29317
Ester Value (mg KOH/g oil)	2.4451	3.1645	9.8407	18.7616	38.236
% Glycerol	0.13365	0.17298	0.53793	1.02558	2.0901

Canola oil has less saturated acids (6%) while the mono-unsaturated acids (62%) are more as compare to poly-unsaturated acids (32%). Results tabulated in table 1 show that prolong storage of oil drastically changes the oil quality by increasing the chain length, oxygen level

and saturation/unsaturation ratio of TAGs. Density and viscosity values were increased by increase in saturation and polymerization of TAGs. Good quality of oil has lower value of density and viscosity.

The unsaturated ingredient in the oil is determined by Saponification value (SV). The oil with low Saponification value has high amount of long chain fatty acids and less unsaturated fatty acid⁸. The SV of fresh oil was comparable to the standard value and decreased on storage and convention heating the oil without carbohydrate, while on repeated frying in presence of piece of carbohydrate, SV of oil was increased. It is clear from the results, the change in SV was less in case of convention heating but in frying experiment, SV rapidly decreased on first frying with carbohydrate piece then increased with repeated frying. Heating the oil reduces the acidity in result less amount of fatty acid remains in oil and SV decreased⁹. Frying oil with carbohydrate increases the amount of saturated fatty acid and an increase was observed in saponification values¹⁰.

The amount of free acids present in oil is measured by acid value (AV). Acid content in oil on heating causes rancidity. Rancidity deteriorates nutritive value of oil which can prevent by addition of antioxidants. Antioxidants react with free radicals and block the formation of fatty acid radicals and terminating the chain reaction¹¹. Acid value for fresh oil was 0.8059 mg KOH/g_{oil} while increased by prolonged storage to 9.423 mg KOH/g_{oil}. Convention heating and frying with carbohydrate leads to decrease in acid value due to the oxidation of fatty acids to peroxides and prolong heating and frying convert these peroxides into secondary oxidation products¹².

The percentage free fatty acid (%FFA) of oil is usually calculated in terms of oleic acid by this relation¹³

$$\% \text{FFA} = \text{Acid value} \times 0.503 \quad (4)$$

Values of %FFA were decreased on conventional heating and frying treatment. Large decrease in %FFA were observed for frying samples.

Ester value is a measure of amount of glyceride present in oil sample, which is saponifiable. Ester value and Percent Glycerol in the oil is calculated by using following relation¹⁴

$$\text{Ester value} = \text{Saponification Value} - \text{Acid Value} \quad (5)$$

$$\% \text{ Glycerol} = 0.0547 \times \text{Ester value} \quad (6)$$

Change in ester values convention heating shows gradual decrease while increase was observed with repeated frying with carbohydrate. Variation in values of % glycerol supported the above results. % glycerol decreased with convention heating while increased with repeated carbohydrate frying.

The amount of active oxygen contained in oil or extent of lipid auto-oxidation during heating and frying is determined by peroxide value (PV). The PV of oil samples ranges from 4.425 - 240.48 meqO₂/Kg. Results show that the PV of fresh oil is changes from 21.52 meqO₂/Kg to 152.066 meqO₂/Kg after long storage due to the increase in oxygen level. The PV was decreased from 240.48 meqO₂/Kg (30°C) to 175.68 meqO₂/Kg (boiling point) in case of convention heating. The range of PV for the repeated fried oil with carbohydrate (potato piece) ranges from 107.39 meqO₂/Kg (first frying) to 4.425 meqO₂/Kg (fifth frying). The PV of the oil was

very high (>10 meq O₂ / Kg) which indicates that the oil is of very low quality. Unsaturated fatty acids easily react with oxygen to form peroxides. High degree of unsaturation is most susceptible to auto-oxidation, involving oxygen that leads to deterioration of oil. At high temperature (particularly at boiling point and at frying), the unsaturated fatty acids present in the oil are unstable and leads to oxidation to form primary products (hydroperoxides) which on further oxidation converts to secondary products low molecular weight like aldehyde, alcohols, ketones, free fatty acids, dimmers, trimmers, polymers and cyclic compounds, which causes decrease in PV values. Decreased in PV indicated that the increased rate of oxidation during convention heating and frying due to break down of double bonds (unsaturation) in fatty acid by oxidation and polymerization^{12,15}.

FT-IR is the simplest and easiest tool for qualitative analysis of samples. The shape, structure and intensity of peaks determined the identity of sample. The spectral bands of the canola oil changes due to the temperature variation and repeated frying experiment with carbohydrate. The oxidation process in the oil reduces the fatty acid proportion which leads to the shift in the position of the spectral bands¹⁶. Figure 1 show the FTIR spectra of canola oil at room temperature, conventional heating at 60°C, at boiling point and first, third and fifth frying with carbohydrate.

A similar signal pattern of spectra was obtained for all oil samples with different intensities. Percentage intensities of all peaks in the spectra was increased with convention heating and repeated frying indicating that the absorbance of the oil was decreased. The peak in the range 3000±150cm⁻¹ was due to the presence of C-H bond in the molecule. Maximum absorbance was observed at 2926.01cm⁻¹ which was due to the symmetric stretching vibration of aliphatic -CH₂ and a peak at 3006cm⁻¹ was assigned to stretching vibration of sp² =C-H¹⁷. Cis =C-H peak was high for non-oxidized oil due to the high proportion of polyunsaturated acyl group and low frequency of mono unsaturated and saturated acyl groups. As the temperature of the oil was increased, the intensity of the peak was decreased due to the breakdown of the cis =C-H bond and formation of hydroperoxide. The peaks obtained in the range of 3000-2840cm⁻¹ was due to the stretching vibration of sp³ -C-H. Presence of cis -C=C- stretching vibration was confirmed by a peak between the ranges 1654.92-1647.21cm⁻¹. This was an indication of presence of unsaturated fatty acid¹⁸. Conjugation can move this peak towards lower frequency and increases the intensity of the peak. Cis olefins show stronger peak as compare to trans olefins¹⁶.

The peaks in the range 1715±100cm⁻¹ is related to the presence of C=O bond (carbonyl group). Another strong band was obtained at 1745.58cm⁻¹ which shows double bond stretching of ester carbonyl of triglycerides (C=O). Non-oxidized oil samples have higher number of ester bond as compare to the oxidized oil. As the oxidation takes place the intensity of the peak changes which shows the formation of peroxides. Three peakes in the range 1260-1000 cm⁻¹ is attributed to the C-O stretching vibration of ester.

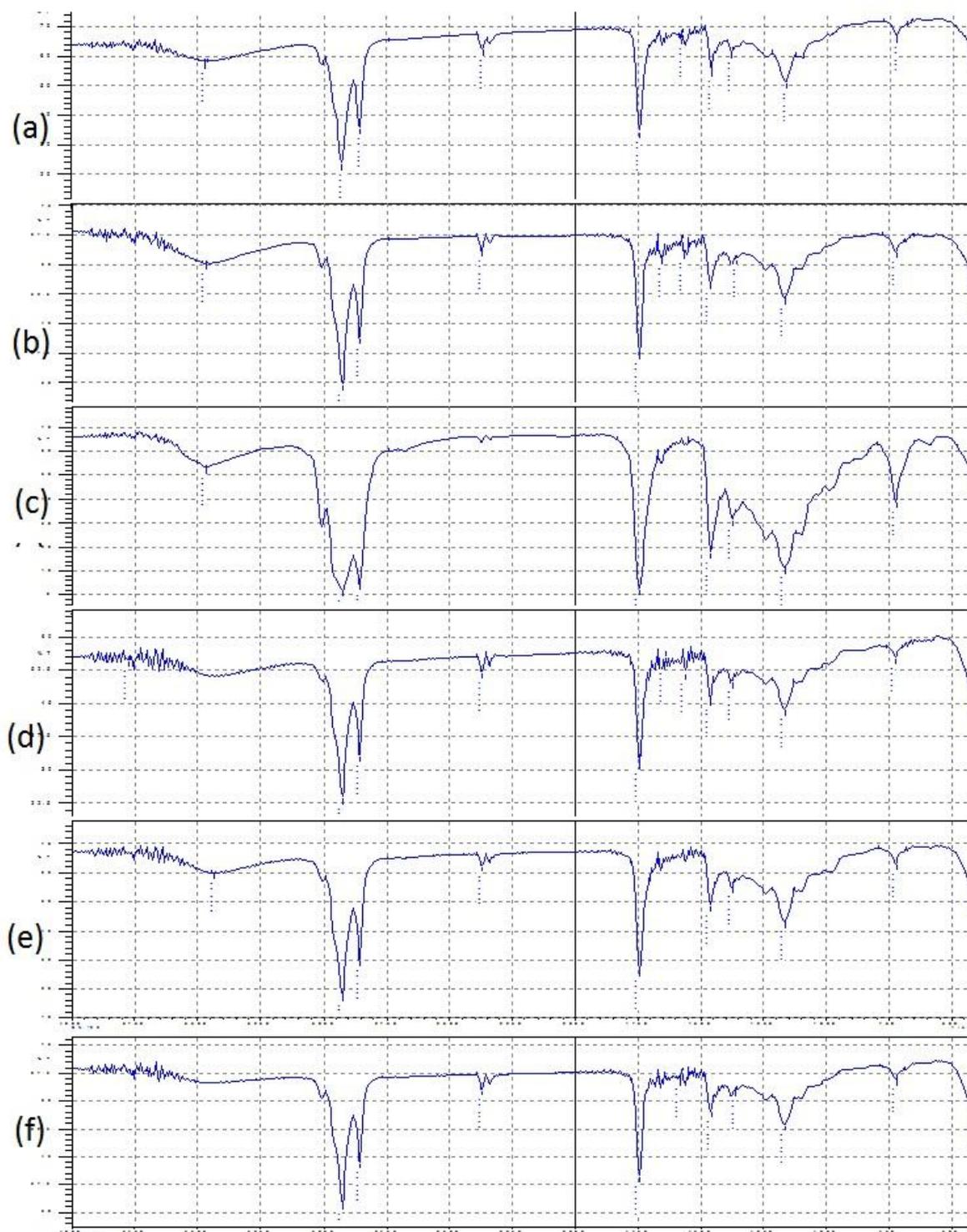


Figure 1 a) FT-IR spectrum of canola oil at (a) room temperature, (b) 60°C, (c) boiling point (d) first frying, (e) third frying, (f) fifth frying

The spectral region at 1458.16-1462.04 and 1359.82-1379.10 cm^{-1} show the peak of bending vibration of CH_2 and CH_3 aliphatic group respectively and favours the increase in the oxidation of oil with temperature. The overlapping of long chain cis $-\text{CH}_2$ bending (rocking) and $-\text{CH}=\text{CH}-$ bending of olefins is shown by the peak at 723.31cm^{-1} . The absence of peak between the range 970-

908cm^{-1} confirmed the absence of trans $\text{CH}=\text{CH}$ unsaturation.

In infrared spectrum, near 1705cm^{-1} appears the peak at boiling point and at all times of frying with carbohydrate which shows the double bond stretching of $-\text{C}=\text{O}$ free fatty acids. In canola oil, presence of sulfur was determined by 1 or 2 broad intense absorption between

the range $2372.44\text{-}2362.80\text{cm}^{-1}$ due to stretching of $\text{X}=\text{C}=\text{S}$.

Broad band at $3469.94\text{-}3452.58\text{cm}^{-1}$ is associated with the stretching vibration of O-H. As the temperature increased, the oxidation process in the oil increases and peroxides are formed which on further heating converts to

secondary products. Additional peak formed at $3753.48\text{-}3750\text{cm}^{-1}$ favours the formation of secondary oxidation products in oil and the band of O-H stretching vibration is merged in the peak of oxidized products. As the concentration of secondary product is increased, deformation of the band takes place and the band is shifted to the higher value of frequency.

Table 4. Degree of chain length and degree of branching of oil samples by FT-IR

	Degree of Chain length		Degree of Branching
	A_{723}/A_{1375}	A_{723}/A_{1460}	A_{1375}/A_{1460}
Conventional Heating			
30°C	0.81296	0.5522	0.6792
60°C	0.8233	0.7294	0.8859
Boiling point	0.8326	0.744	0.8937
Potato Frying			
1 st Frying	0.8149	0.6729	0.8257
3 rd Frying	0.8585	0.7419	0.8642
5 th Frying	0.8673	0.7800	0.8993

Intensities of the peaks in FTIR spectrum was further used to obtain the useful information regarding the viscous nature of the oil by calculating the degree of chain length and degree of branching of fatty acids of the oils. Absorbance of CH_3 symmetric deformation at 1375cm^{-1} and $\text{CH}_2 + \text{CH}_3$ symmetric deformation at 1460cm^{-1} were used to calculate the degree of branching

(A_{1375}/A_{1460}) and degree of chain length (A_{723}/A_{1375} or A_{723}/A_{1460}) is the ratio of absorbance of CH_2 rocking at 723cm^{-1} with the absorbance of CH_3 or $\text{CH}_2 + \text{CH}_3$ symmetric deformation¹⁹⁻²⁰. The values of degree of chain length and degree of branching of oil samples after conventional heating and potato frying reported in the Table 4 revealed that these values were increased for both treatments. Frying experiment has greater values of degree of branching and degree of chain length as compare to the conventional heating. Viscosity have direct relation with the saturation, chain length and branching of fatty acids. From the above results, it is concluded that oil become thicker and rancid with heating and frying treatment.

CONCLUSION

Cooking and frying different food items in oil is routine practice at domestic and commercial level. For this purposes, same oil is reused many times. The results of present investigation declare that the prolong storage, convention heating and repeated frying with potato piece deteriorates the oil quality and shifts oil towards its lowest nutritive value which leads to the formation of non-volatile compounds that increases health risks. So, there is a need to take necessary steps to re-enhance the nutritive value of used oil to reduce the risk of health hazards.

Conflict of Interest

Authors have declared that no competing interests exist.

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