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Successive deep-Frying impact on the physicochemical properties of Himalayan Cheura oil

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The seed oil derived from *Diploknema butyracea* (Roxb.) H. J. Lam has considerable significance in the Western Himalayan regions of India and Nepal. It serves as a vital resource for livelihoods and is widely employed for cooking and ethno-medicinal purposes. Reusing oil for frying is a common practice during cooking. Frying alters the composition and quality of oil. This study, for the first time, assesses the physicochemical properties of Cheura oil after three repeated frying cycles using potatoes and chicken meat. Various physicochemical parameters, including visual appearance, moisture, viscosity, Fourier transform infrared spectroscopy analysis, and chemical parameters (peroxide, acid, iodine, saponification, and ester values) were evaluated. Comparisons were made between unfried oil and three frying cycles of potatoes and chicken. Results revealed slight refractive index and viscosity changes, while spectral analysis showed significant functional group alterations. Also, colour intensity, moisture, saponification (12.06-14.025 mg KOH/g), acid (2.29-3.87 mg KOH/g), peroxide (6.45-16.45 meqO_2/kg), and ester values (8.85–13.65 mg KOH/g) increased, while iodine (22.32 to -11.62 g/100g) decreased, indicating progressive quality deterioration over frying cycles. Overall, Cheura oil is suitable for frying; however, repeated use degrades its quality.

Keywords: Diploknema, Frying, FTIR, Physicochemical, Rheology

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Introduction

Vegetable oils consist of triacylglycerol molecules, comprising fatty acids attached to a glycerol backbone, and are obtained from plant seeds for food, pharmaceutical, and industrial applications. Although vegetable oils are predominantly utilised for culinary purposes (such as cooking oils and margarine), they possess significant potential for diverse applications based on the physicochemical characteristics[1]. Edible oils constitute a fundamental component of the human diet, providing a rich amount of lipids, fatty acids, proteins, sterols, vitamins, and antioxidants, which contribute to energy provision, nutritional needs, tissue healing, and cellular regeneration[2].

Cheura oil is an edible vegetable oil derived from the mature seeds of *D. butyracea*. It is a native Himalayan plant mostly found in India, Nepal, Bhutan, and China, with significant medicinal and economic importance for many ethnic populations in the western Himalayan region. The Indian butter tree, *D. butyracea*, often referred to as Cheura or Chyuri, and Phulwara butter, is extensively utilised by indigenous communities for subsistence, cultural, economic, and therapeutic purposes[3]. The different components of the Cheura tree have multiple uses and serve as a valuable source of oil or butter, honey, and jaggery. The primary component of Cheura seeds is its lipid (Cheura fat), utilised by the ethnic community as a vegetable cooking oil and as a massage oil for rheumatism, headaches, acne, and boils. Cheura oil mostly consists of triglycerides, which include palmitic acid, oleic acid, stearic acid, and linoleic acid[3].

Frying oil or butter at high temperatures in the presence of air leads to substantial alterations in their physicochemical properties. These alterations arise from chemical reactions like oxidation, hydrolysis, isomerization, and polymerization that occur during frying[4]. Repeated frying in the same oil leads to a

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substantial alteration in the composition of fatty acids, resulting in oil quality degradation and serious health risks for consumers. Studies have reported a marked reduction in polyunsaturated fatty acids (PUFA) level[5]. Various factors influencing the deep-frying process include the addition of fresh oil, frying conditions, oil quality, food materials, fryer type, antioxidants, and oxygen concentration[6]. The quality of oils and their effects on repeated frying can be assessed by various physicochemical analytical evaluation procedures. Numerous prior studies have examined various vegetable oils to assess the quality post-frying, indicating a decrease in quality that renders the oil unsuitable for consumption[7–9].

Although studies have examined the impact of deep-frying on various edible oils, no systematic investigation has yet been performed on Cheura oil on repeated frying. Cheura oil holds significant cultural, nutritional, and economic value in the Western Himalayan region, where it serves as a traditional cooking medium and an important source of livelihood for local communities. Given its widespread culinary use and unique regional importance, the present study represents the first comprehensive assessment of the physicochemical changes and quality degradation in Cheura oil during successive deep-frying cycles. The findings aim to provide critical insights into its thermal stability and potential implications for public health, culinary practices, and sustainable utilisation.

Materials and Methods

Lipid collection

The Himalayan Cheura oil was obtained from the mature seeds of *D. butyracea*, procured from a self-help group in the Pithoragarh district of Uttarakhand and maintained at ambient temperature. The seed sample was authenticated by the Pharmacopoeia Commission of Indian Medicine and Homeopathy, Ghaziabad (authentication number ULRNTC1131224000000190F).

Chemicals

All the chemicals used in this study were of analytical grade (AR) and were procured from Central Drug House (CDH, New Delhi, India) and LOBA Chemie (Maharashtra, India).

Frying process

The oils were fried according to the prior procedure[10], with the addition of chicken meat during the frying process. Chopped potatoes and chicken were fried in the same oil over three consecutive sessions.

Physical analysis of the oil sample

Visual examination

The visual examination test serves as the initial step in the preliminary evaluation of frying oil quality. The colour changes of the oil were visually observed after each frying session.

Moisture content

The moisture content of the samples was determined using a Mettler-Toledo semi-automated moisture analyser. Exactly 1 g of the sample was weighed and placed on the metallic plate, then heated at 110±2°C for 10 minutes.

Viscosity analysis

The rheological properties of Cheura lipid were analysed using an Anton Paar viscometer, following a modified protocol. Measurements were conducted at a constant temperature (± 0.1°C) with shear rates ranging from 0.1 to 100 s^{-1}.

Refractive index

The refractive index of the sample was measured using an Abbe refractometer. A small amount (2-3 drops) of the sample was placed in the prism compartment and allowed to settle for a few minutes. The refractive index was then determined by reading the telescope scale, with *n*-butanol used as a reference for calibration.

Spectral analysis

The spectra were observed using a Bruker ALPHA Fourier transform infrared spectroscopy (FTIR) II spectrometer equipped with an ECO-ATR module. The sample was carefully applied to the optics using a spatula and scanned in reflectance mode over a spectral range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

Chemical analysis of oil samples

Peroxide value

The peroxide value measures the concentration of molecules in lipid samples that oxidise and convert potassium iodide (KI) into iodine. About 1 g of oil was dissolved in a 3:2 acetic acid and chloroform mixture, followed by the addition of 1 mL saturated KI. The amount of iodine liberated from KI is then

quantified by titration with 0.01N sodium thiosulfate ($Na_2S_2O_3$), using a 1% starch indicator. A control trial was performed for comparison[11].

$$PV = \frac{(s-b) \times N \times 1000}{W}$$

Where, *PV* = peroxide value, *s* = volume of titer for sample, *b* = volume of titer for blank, *N* = Normality of $Na_2S_2O_3$, *W* = Weight of the sample (g).

Acid value

The acid value determines the free fatty acids present in a fat or oil sample and is determined through titrimetric analysis. Approximately 1 g of the oil sample was dissolved in neutralised alcohol and titrated with 0.1 N potassium hydroxide (KOH) solution using phenolphthalein as an indicator[12].

$$AV = \frac{56.1 \times N \times V}{W}$$

Where, *AV* = acid value, *N* = Normality of KOH, *V* = volume of KOH used in titration, *W* = Weight of the sample (g).

Iodine value

The iodine value of Cheura oil was determined using the Hanus method[13]. Approximately 1 g of oil was dissolved in 10 mL of carbon tetrachloride in a conical flask, followed by the addition of 30 mL of Hanus solution. The mixture was incubated in darkness for 45 minutes with periodic agitation. Subsequently, 10 mL of 15% KI solution and 100 mL of distilled water were added. The iodine was titrated with 0.1N $Na_2S_2O_3$ using a 1% starch indicator until the blue colour disappeared. The volume of $Na_2S_2O_3$ used was recorded, and a blank titration was performed for comparative assessment.

$$IV = \frac{(B-A) \times N \times 0.127 \times 100}{W}$$

Where, *IV* = iodine value, *B* = mL of 0.1 N$Na_2S_2O_3$ required by blank, *A* = mL of 0.1 N$Na_2S_2O_3$ required by sample, *N* = Normality, *W* = Weight of the sample (g).

Saponification value

The saponification value was determined by adding 25 mL of 0.5 N alcoholic KOH to 1 g of Cheura lipid in a round-bottom flask. The mixture was heated under reflux for 30 minutes to ensure complete saponification of the sample. A blank sample, prepared without the oil, was processed in the same manner. After saponification, the solution was cooled, phenolphthalein was added as an indicator, and the mixture was titrated with 0.5 N hydrochloric acid (HCl)[14].

$$SV = \frac{(B-T) \times N \times 56.1}{W}$$

Where, *SV* = saponification value, *B* = volume of titer for blank, *T* = volume of titer for sample, *N* = Normality of KOH, 56.1 = equivalent weight of KOH, *W* = Weight of the sample (g).

Ester value

The ester value represents the amount of KOH required to hydrolyse the esters present in a specific quantity of oil or fat sample[15]. The ester value is calculated using the following formula:

$$EV = SV - AV$$

Where, *EV* = Ester value, *SV* = Saponification value, *AV* = Acid value.

Statistical analysis

The statistical analysis of data was done using GraphPad Prism 10.0 software. All the values were presented as Mean ± Standard Deviation (SD). Comparisons among groups were done by using two-way ANOVA (Analysis of Variance) followed by Dunnett's Multiple comparison, and comparisons within groups were done by using Ordinary one-way ANOVA followed by Tukey Multiple comparison. *P values* less than 0.05 were considered indicative of significance.

Results and Discussion

Physical analysis of the oil samples

Visual examination

Colour is a crucial parameter for preliminary examination[7]. The colour change results from chemical reactions, including oxidation and polymerisation. The darkening of oil after frying may also arise from the Maillard reaction, which involves interactions between components and nutrients in food, such as sugars and amino acids[16]. The colour of the unfried Cheura oil depicted in Fig. 1a appeared pale whitish, which transformed to a deep yellow following the initial frying with Meat-fry (M-fry) I, II, and III illustrated in Fig.1c. In contrast, when potatoes were fried, the colour shifted to pale yellow in Potato-fry (P-fry) I, subsequently diminishing in intensity in P-fry II and P-fry III compared to the first frying round, as shown in Fig. 1b. The carbon constituents in the potato or meat samples may migrate into the oil during frying, perhaps imparting the distinctive colour to the oil samples. The sample comprises unsaturated fatty acids that undergo heat oxidation and

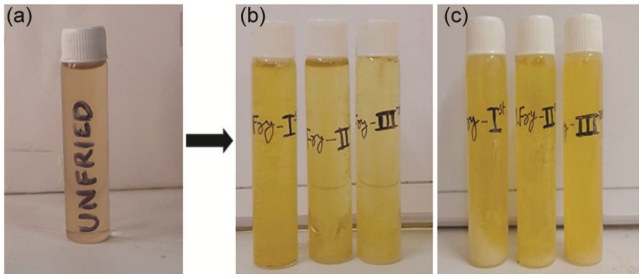

Fig. 1 — Effect of frying Cheura oil on colour. (a) Unfried Cheura oil, (b) Potato fried, and (c) Chicken meat fried.

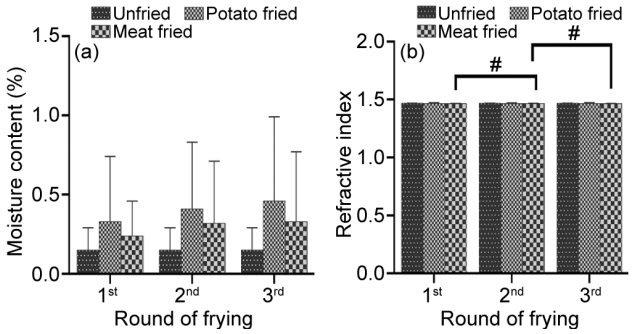

Fig. 2 — Effect of frying Cheura oil on (a) Moisture content, and (b) Refractive index. All values are expressed as Mean ± SD; N = 3 in each round of frying, two-way ANOVA followed by Dunnett's multiple comparison was applied for statistical analysis. *P values:* $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ when results of different rounds of frying were compared among different groups of frying.

polymerisation, resulting in the formation of non-volatile compounds or a potential increase in the linoleic content of the oil[17]. During deep frying, pigments including non-volatile chemicals and carbonyl compounds are generated and aggregate in the oil, resulting in a deeper colouration of the oil post-frying[18].

Moisture content

Moisture content is a critical metric for assessing oil quality and confirming oil dryness[19]. The moisture content is influenced by temperature, frying duration, and oil composition[20]. Fig. 2a illustrates the moisture content of unfried Cheura oil and the effects of repeated frying with potato and meat samples. The moisture content of the sample prior to frying (unfried Cheura oil) was determined to be 0.15±0.14%, which appears to have increased following further frying with potato and meat. An increase in the moisture content of the sample was observed, rendering the oil sample more vulnerable to hydrolytic breakdown[20]. Frying food materials results in water loss; hence, when potatoes and meat were fried, moisture was leached into the oil, potentially increasing the oil's moisture content[21]. The meat also contains a significant amount of collagen, which may influence the moisture content during the frying process in oil[22]. The alteration in the moisture content of the sample appears to be influenced by temperature, as elevated temperatures facilitate moisture evaporation, thereby preserving oil quality. However, excessively high temperatures may result in oxidation and degradation of the oil, adversely impacting its quality and flavour[8].

Refractive index

The refractive index quantifies the purity and quality of oil by comparing the velocity of light in a vacuum to that in oil. This physical characteristic is extensively employed in numerous applications for substance identification, characterisation, and purity evaluation, with every substance exhibiting a unique refractive index[23]. The refractive index of fats and oils indicates the typical level of unsaturation in these substances and is utilised to monitor the progress of reactions like hydrogenation and isomerization[24]. Fig. 2b illustrates the refractive index of the oil sample before and after frying. Prior to frying, the refractive index of the sample was recorded as 1.469±0.001. After frying, the refractive index decreased in successive P-fried and M-fried oils. The slight increase in refractive index observed in Cheura oil after frying meat may be due to increased opaqueness caused by the solubilization of pigments like melanoidins or the increased viscosity of the oil due to polymerization[25]. However, significant changes ($P<0.05$) in the refractive index were observed between M-fry I and M-fry II oils, as well as between M-fry II and M-fry III oil samples.

Viscosity

Viscosity serves as a measure for assessing the physical changes in butter or oils, as these substances are composed of triglycerides. The configuration of fatty acids on the glycerol backbone is intrinsically linked to their chemical properties, including chain length and the extent of saturation or unsaturation, which affect viscosity[10]. Cheura oil is a triglyceride mixture that exhibits viscosity variations depending on its triglyceride composition. Fig. 3a illustrates that the viscosity of Cheura oil diminishes as the shear rate increases, indicating a more pronounced reduction at elevated shear rates, which is indicative of shear thinning behaviour. This behaviour indicates that Cheura oil exhibits non-Newtonian fluid characteristics. The connection between shear rate and shear stress depicted in Fig. 3b indicates a non-linear increase in shear stress

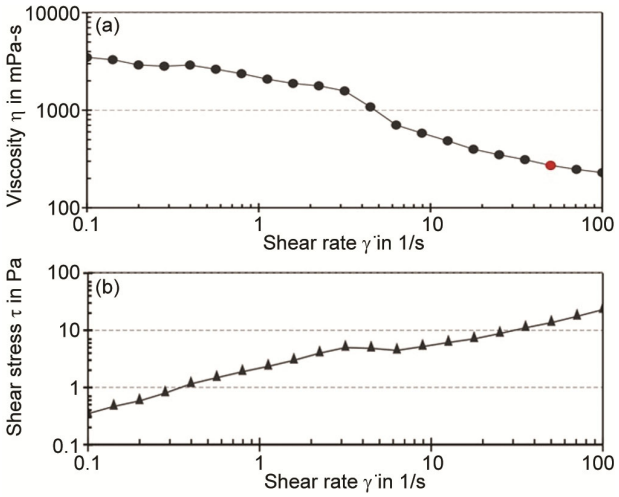

Fig. 3 — Rheological property of unfried Cheura oil. Effect of shear rate on (a) viscosity, and (b) shear stress.

with varying shear rate, which suggests that Cheura lipid exhibits pseudoplastic, non-Newtonian fluid characteristics[26].

The viscosity of Cheura oil after frying with potato (Fig. 4a-f) and meat (Fig. 4g-l) demonstrated negligible variation across different shear rates; however, the total viscosity of both the P-fry and M-fry oil was markedly elevated compared to unfried oil. This indicates that frying causes compositional alterations, including oxidation, polymerisation, and the development of large molecular aggregates or cross-linked compounds, as well as food residues and elevated free fatty acid content, resulting in increased oil viscosity with each frying cycle[27]. The rheological studies indicate that the viscosity of oil is predominantly affected by its molecular configuration, decreasing with elevated unsaturation

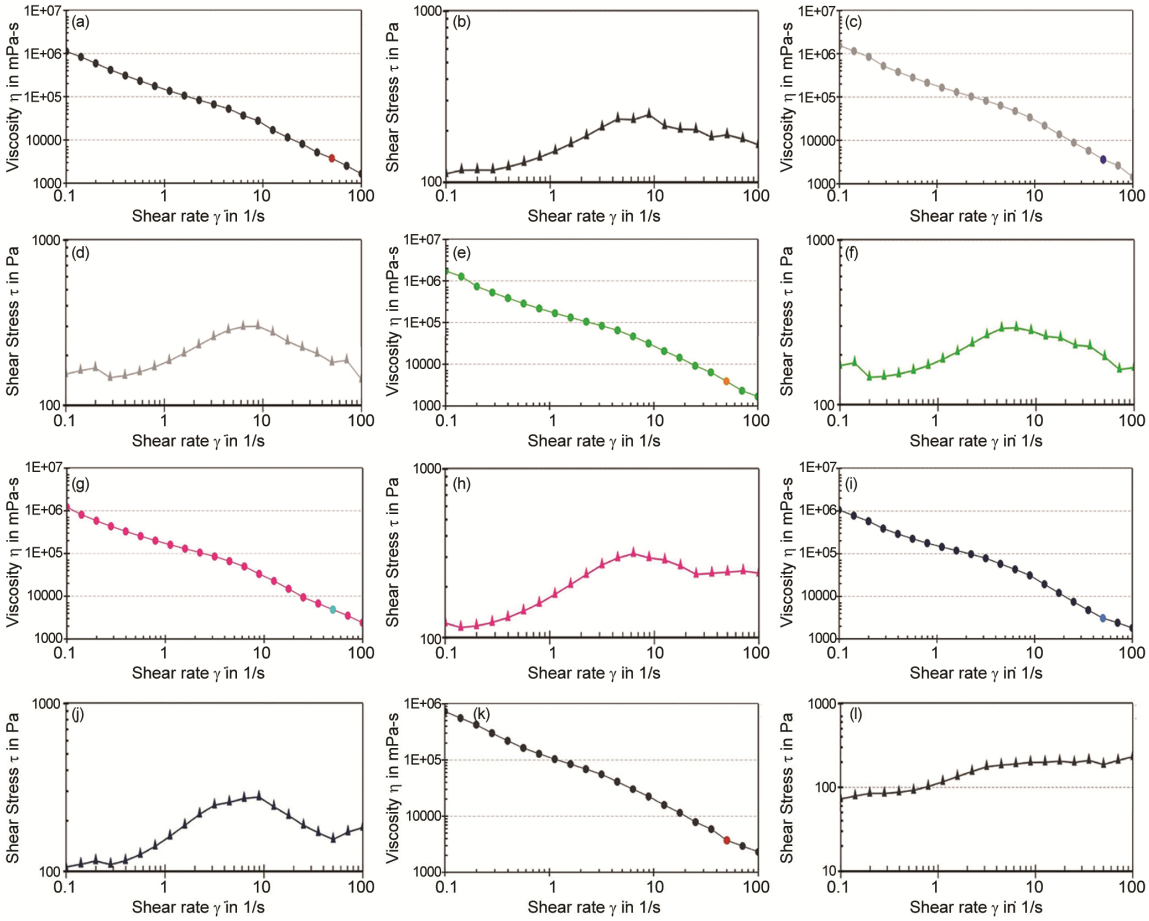

Fig. 4 — Rheological properties of potato and meat fried and Cheura oil. (a) Effect of shear rate on viscosity of P-fry I oil, (b) Effect of shear rate on shear stress of P-fry I oil, (c) Effect of shear rate on viscosity of P-fry II oil, (d) Effect of shear rate on shear stress of P-fry II oil, (e) Effect of shear rate on viscosity of P-fry III oil, (f) Effect of shear rate on shear stress of P-fry III oil, (g) Effect of shear rate on viscosity of M-fry I oil, (h) Effect of shear rate on shear stress of M-fry I oil, (i) Effect of shear rate on viscosity of M-fry II oil, (j) Effect of shear rate on shear stress of M-fry II oil, (k) Effect of shear rate on viscosity of M-fry III oil, and (l) Effect of shear rate on shear stress of M-fry III oil.

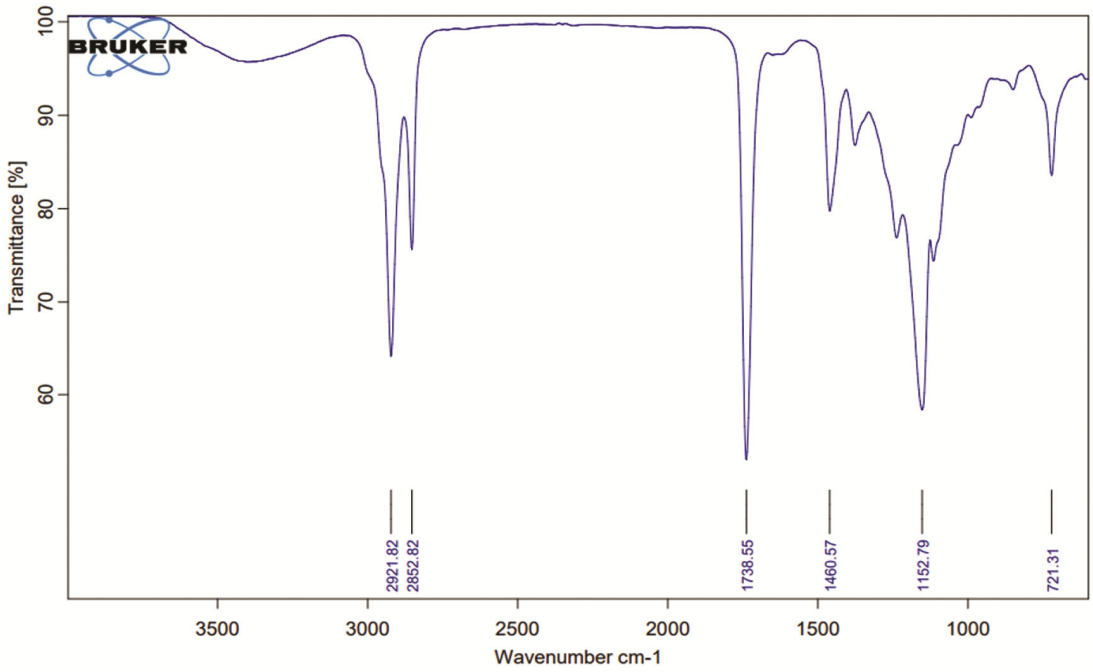

Fig. 5 — FTIR spectrum of unfried Cheura oil.

levels in fatty acids, while increasing with longer chains of saturated fatty acids. This is attributable to the stiffness conferred by Pi (π) bonds, which restrict rotation around carbon-carbon bonds; longer chains facilitate flow and decrease viscosity, possibly due to reduced friction among fatty acid chains[28]. Viscosity at higher temperatures enhances molecular motion, decreases intermolecular interactions, and facilitates the sliding of liquid layers past one another, leading to lower viscosity[29].

Spectral analysis

The assessment of spectra by FTIR spectroscopy plays a crucial role in the analysis of oils and fats, as the intensity of the spectral bands is closely correlated with the composition and concentration in the sample[10]. Mid-infrared spectra are frequently utilised to characterise edible oils and fats because of their unique differences in intensity and the exact frequencies at which maximal absorbance or transmittance is observed. These differences are contingent upon the distinct composition and characteristics of the compounds within the sample. The intensity peaks of functional groups have been detected in the unfried Cheura oil, as illustrated in Fig. 5. These peaks correspond to distinct functional group. The associated functional group in unfried Cheura oil is presented in Table 1. The peaks at 2921.82 cm^{-1} and 2852.82 cm^{-1} are attributed to C-H stretching, whereas the peak at 1738.55 cm^{-1} corresponds to C=O (ester) stretching[30]. The peaks at 1152 cm^{-1}, 1460 cm^{-1}were attributed to C-O and C-H bending, while the peak at 721.31 cm^{-1} was related to bending CH_2[10].

The spectral analysis of P-fried oils following successive frying rounds, as illustrated in Fig. 6a-c, revealed significant peaks at 650.92 cm^{-1}, 1718.63 cm^{-1}, and 781.35 cm^{-1}, corresponding to the stretching of halo compounds (C-Br), stretching of carboxylic acids (COOH), and bending of alkenes (C=C), respectively[30]. In the P-fry oils, distinct peaks were identified; specifically, in P-fry I oil, a peak at 3037.14 cm^{-1} indicates the existence of C–H stretching, while another peak at around 2618.47 cm^{-1} corresponds to thiol (S-H) stretching[30]. In P-fried II oil, the peaks at 1777.32 cm^{-1}, 1718.63 cm^{-1}, and 1073.91 cm^{-1} correspond to C=O carbonyl stretching, conjugated alkene (C=C) stretching, and primary alcohol (C-O) stretching, respectively. In the P-fried III oil, distinct peaks were identified at 2205.29 cm^{-1}, 2067.86 cm^{-1}, 1990.64 cm^{-1}, 1070.45 cm^{-1}, and 734.66 cm^{-1}, potentially corresponding to alkyne (C≡C) stretching, azide (N=N=N), isothiocyanate (N=C=S), =C–H bending, and mono-substituted benzene derivatives (C-H), respectively[31]. The distinct peaks indicate the presence of various functional groups in the oils, possibly resulting from compositional \

Table 1 — FTIR analysis and changes in functional group assignments of Cheura oil before and after frying

Unfried Cheura oil		P-fry I		P-fry II		P-fry III		M-fry I		M-fry II		M-fry III	
Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.
2921.82	C-H stretching	3733.67	O–H (stretching)	3405.82	O–H (stretching)	3042.17	Carboxylic acid, alcohol, alkene (stretching)	3723.54	O–H (stretching)	3845.64	O-H (Stretching)	3862.38	O–H (stretching)
2852.82	C-H stretching	3651.01	O–H (stretching)	2874.91	Aldehyde (stretching)	2951.77	Alkane (stretching)	3678.96	O–H (stretching)	3492.93	O-H (Stetching)	3735.76	O–H (stretching)
1738.55	C=O stretching	3271.46	O–H stretching	1777.32	C=O carbonyl (stretching)	2935.28	Alkane (stretching)	3553.04	O–H (stretching)	2905.55	Alkane (stretching)	3408.21	Primary amine (stretching)
1460.57	Alkane bending C- H	3191.70	O–H (stretching)	1718.63	Carboxylic acid (stretching)	2786.17	Alkane (stretching)	3497.07	O–H (stretching)	2309.04	Carbon dioxide (stretching)	2901.63	Alkane (stretching)
1152.79	C-O (stretching)	3037.14	C–H stretching	1637.83	Conjugated alkene (stretching)	2701.02	Aldehyde (stretching)	3364.43	O–H (stretching)	1775.18	C=O (stretching)	2386.15	Carbon dioxide (stretching)
721.31	C=C (bending)	2785.24	Aldehyde (stretching)	1370.03	Alkane (bending)	2508.09	Carboxylic acid (stretching)	3268.49	O–H (stretching)	1725.29	C=O (stretching)	2310.10	Carbon dioxide (stretching)
		2618.47	Thio l (stretching)	1142.44	Aliphatic ether(stretching)	2355.55	Carbon dioxide (stretching)	3211.62	O–H (stretching)	1397.24	Alkane (bending)	1773.66	C=O (stretching)
		2314.98	Carbon dioxide (Stretching)	1073.91	Primary alcohol (stretching)	2205.29	Alkyne (stretching)	3114.02	O–H (stretching)	1177.91	Tertiary alcohol (stretching)	1721.51	C=O (stretching)
		1754.12	Conjugated anhydrid (stretching)	653.70	Halo compound (stretching)	2147.17	Azide (stretching)	2951.33	Alkane, amine salt, alcohol, carboxylic acid (stretching)	1097.82	Secondary alcohol (stretching)	1517.71	Nitro compound (stretching)
		1554.21	Nitro compound (stretching)			2067.86	Isothiocyanate (stretching)	2885.92	Alkane, amine salt, alcohol, carboxylic acid (stretching)			1400.16	Carboxylic acid (bending)

(*Contd.*)

Table 1 — FTIR analysis and changes in functional group assignments of Cheura oil before and after frying (*Contd.*)

Unfried Cheura oil		P-fry I		P-fry II		P-fry III		M-fry I		M-fry II		M-fry III	
Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.	Wave no. cm^{-1}	Functional group.
		1151.83	C–O stretching			1990.64	Isothiocyanate (stretching)	2717.28	Aldehyde (stretching)			1182.44	Sulfonate (stretching)
		901.21	Alkene (bending)			1546.73	Nitro compound (stretching)	2565.29	Thiol (stretching)			1093.94	Secondary alcohol (stretching)
		730.98	Alkene (bending)			1494.97	Alkane (bending)	2423.22	Carbon dioxide (stretching)			706.24	Alkene (bending)
		650.92	Halo compound (stretching)			1070.45	Sulfoxide (stretching)	2360.67	Carbon dioxide (stretching)			632.20	Halo compounds (bending)
						1047.19	C–O (stretching)	2304.30	Carbon dioxide (stretching)				
						985.94	Alkene (bending)	2003.57	Isothiocyanate (stretching)				
						797.51	Alkene (bending)	1853.37	C=O (stretching)				
						781.39	Alkene (bending)	1767.95	Anhydride (stretching)				
						734.66	=C–H (bending)	1714.13	Aliphatic ketone, carboxylic acid (stretching)				
						656.01	Halo compound (stretching)	1521.36	Nitro compound (stretching)				
						624.94	Halo compound (stretching)	1347.02	C–H (bending)				
						613.73	Halo compound (stretching)	1230.41	Ester C–O (stretching)				
						604.59	Halo compound (stretching)	1061.46	C–O (stretching)				
								1003.76	C–O (stretching)				
								842.65	Halo compound (stretching)				
								723.24	Alkene (bending)				
								629.97	Halo compound (stretching)				

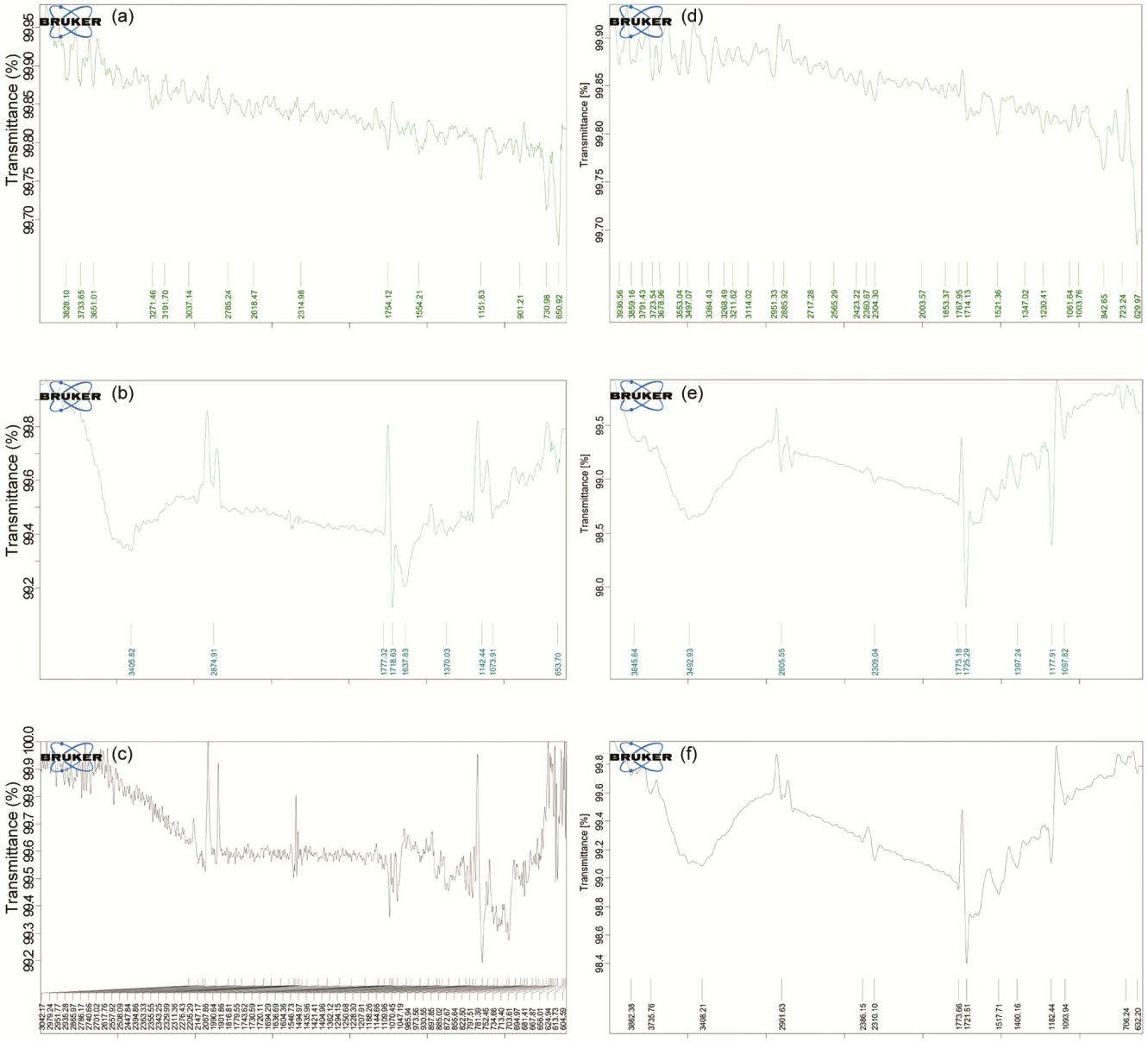

Fig. 6 — FTIR spectrum of the potato and meat fried oil sample on different rounds of frying. (a) P-fry I oil, (b) P-fry II oil, (c) P-fry III oil, (d) M-fry I oil, (e) M-fry II oil, and (f) M-fry III oil.

changes during the frying process. The spectrum analysis of M-fried oils during successive frying cycles is illustrated in Fig. 6d-f. The M-fried oils exhibited analogous peak bands in all samples at 3723.54 cm^{-1}, 3678.96 cm^{-1}, 3497.07 cm^{-1}, 3114.02 cm^{-1}, 2951.33 cm^{-1}, and 2885.92 cm^{-1}, attributed to the presence of O-H stretching vibrations[32]. Additional peaks in M-fry I were detected at 3845.64 cm^{-1} and 3492.93 cm^{-1}, whereas M-fry II and M-fry III exhibited analogous OH stretching peaks at 3862.38 cm^{-1} and 3735.76 cm^{-1}, respectively. The peaks at 2951.33 cm^{-1} and 2885.92 cm^{-1} in M-fry I, 2905.55 cm^{-1} and 1397.24 cm^{-1} in M-fry II, and 2901.63 cm^{-1} in M-fry III were all attributed to alkane (C-H) stretching[33]. A significant peak at 2717.28 cm^{-1} in M-fry I and 1773.66 cm^{-1} in M-fry III was linked to aldehyde (CHO) stretching. However, M-fry II did not display any peaks associated with aldehyde groups, signifying a difference in the chemical composition of the M-fry oils[33].

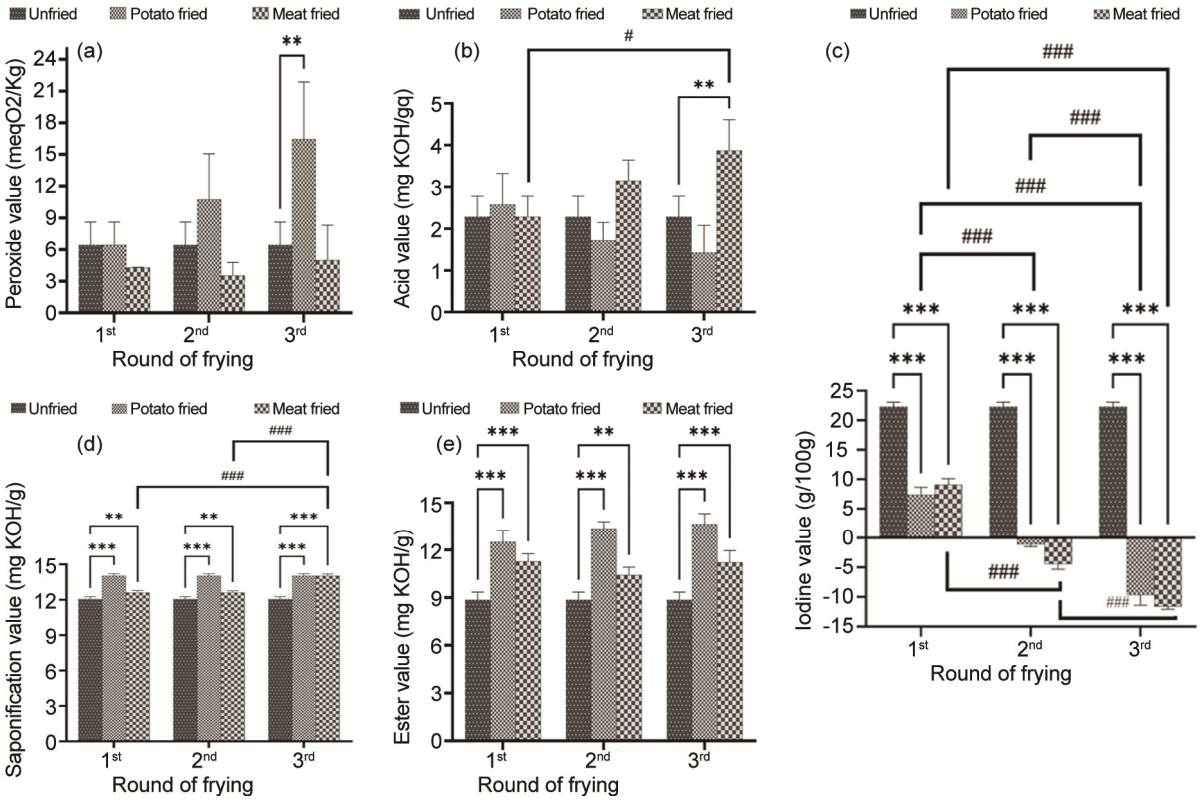

Fig. 7 — Effect of frying Cheura oil on (a) Peroxide value, (b) Acid value, (c) Iodine value, (d) Saponification value, and (e) Ester value. All values are expressed as Mean ± SD; N = 3 in each round of frying, two-way ANOVA followed by Dunnett's multiple comparison was applied for statistical analysis. *P values*: $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ when results of different rounds of frying were compared among different groups of frying. Ordinary one-way ANOVA followed by Tukey multiple comparison was applied for statistical analysis. *P values*: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when results of different rounds of frying were compared within different groups of frying.

Chemical analysis of oil samples

Peroxide value

The peroxide value quantifies the milli equivalents or millimoles of hydroperoxides per kilogram of oil (meqO_2/kg of oil). Peroxide value is employed to assess the quality of oil by detecting alterations in the primary oxidation of fats and oils caused by molecular oxygen species, including hydroperoxide[34]. It quantifies the degree of rancidity reactions that have occurred due to processing, storage, and environmental conditions[10]. The peroxide value of fresh oil should be less than 1 meqO_2/kg, indicating high quality, whereas a value exceeding 10 meqO_2/kg denotes rancidity, signifying poor quality and unfitness for usage[35]. Fig.7a presents the peroxide value of unfried Cheura oil and its repeated frying with potatoes and chicken meat.

In our study, the peroxide value of unfried Cheura oil was 6.45±1.35 meqO_2/kg. After repeated frying, the peroxide value of unfried Cheura oil remained unchanged at 6.45 meqO_2/kg. However, during the first frying cycle (P-fry I), no significant change was observed, with a peroxide value of approximately 6.4±2.15 meqO_2/kg. In the second frying cycle (P-fry II), the peroxide value increased to 10.75±4.3 meqO_2/kg, and further escalated to 16.45±5.4 meqO_2/kg in the third frying cycle (P-fry III). This indicates a significant increase in peroxide value over three frying cycles with potatoes, suggesting that the oil undergoes peroxidation upon repeated frying, potentially compromising its quality and rendering it unsuitable for consumption[36]. A comparison between unfried Cheura oil and P-fried-III oil indicated a significant increase ($P<0.01$) in the peroxide value. The rise in peroxide value is also related to the development of deleterious compounds such as acrylamide and polycyclic aromatic hydrocarbons. The thermal degradation of fatty acids during frying results in the production of peroxides. As the level of breakdown increases, the peroxide concentration increases accordingily[37].

The impact of frying chicken meat in Cheura oil demonstrated a reduction in peroxide value, with M-fry I measuring 4.30±0.1 meqO_2/kg. A further decline

was noted in M-fry II oil, which recorded around 3.54±1.24 meqO_2/kg. However, with additional frying during the third cycle, M-fry III exhibited an increase in peroxide value to 5.01±3.30 meqO_2/kg, in comparison to M-fry I and M-fry II oils. The total outcome of frying chicken meat in Cheura oil demonstrated a reduction in peroxide value compared to unfried Cheura oil. The observed reduction in peroxide value during chicken frying with Cheura oil highlights its potential as a suitable option for frying applications, especially for chicken. The abrupt reduction in peroxide value following the first fry may be attributed to the instability of peroxides generated during heating at elevated temperatures, which subsequently transform into carbonyl and aldehyde compounds, leading to a decrease in peroxide value[36]. The progressive decline in peroxide value during the initial frying cycles may also indicate that frying protein and fat-rich chicken exerts less oxidative stress on the Cheura oil compared to carbohydrate-rich foods like potatoes.

Acid value

The acid value quantifies the concentration of carboxylic acid present. Deep fat frying is a prevalent culinary technique wherein food is immersed in oil at temperatures above 150°C[7,36]. Regular usage of oil for deep frying degrades its quality. The triacylglycerol molecule found in oil undergoes several chemical reactions, including hydrolysis, oxidation, and polymerization[38]. Frying induces the oxidation of oil, and after a period, at a specific concentration, the rates of polymerisation and breakdown increase, resulting in a substantial production of free fatty acids that raise the acid value[26]. The elevation of free fatty acid concentration and the reduction of oil unsaturation signify oxidation and chemical alterations in oils subjected to repeated heating[36].

The average acid value of unfried Cheura oil and the effects of repeated frying with potatoes and meat are illustrated in Fig. 7b. The unfried Cheura oil exhibited an acid value of 2.29±0.49 mg KOH/g. However, when meat was fried in Cheura oil three times at intervals, the acid value after the initial frying in M-fry I was 2.29±0.49 mg KOH/g, whereas in M-II fry, the acid value increased to 3.15±0.49 mg KOH/g. The rise in acid value signifies that following frying, the quantity of free fatty acids has escalated while the oil's unsaturation has diminished. Following the third frying in M-fry III, the acid value increases to 3.87±0.74 mg KOH/g, indicating a rise in FFA and a reduction in the unsaturation of Cheura oil. The sample with the shortest frying duration possesses a larger concentration of mono unsaturated fatty acids (MUFA), facilitating slower oxidation[26].

The sample subjected to prolonged frying exhibits a greater concentration of PUFA, resulting in diminished stability and a more rapid elevation in acid value due to enhanced oxidation susceptibility[26]. Upon frying potatoes in Cheura oil three times at successive intervals, the acid value recorded after the initial frying in P-fry I was 2.58±0.74 mg KOH/g, which is elevated compared to the unfried Cheura oil. Subsequently, in P-fry II, the acid value decreased to 1.72±0.43 mg KOH/g, and after the third frying in P-fry III, the acid value further decreased to 1.43±0.65 mg KOH/g. This results from extended heating at elevated temperatures that transform free fatty acids into esters and other compounds. Additionally, potatoes emit moisture, which may result in the evaporation of free fatty acids[20]. Thermal degradation of the frying medium leads to polymerisation, oxidation, and hydrolysis, which thereby decreases the oil's acid value[20]. A significant increase in acid value was observed when unfried Cheura oil was compared to M-fry III oil ($P<0.01$) and between M-fry I and M-fry III oils ($P<0.05$). Conversely, when meat is fried in Cheura oil for extended durations, an increase in acid value is noted with prolonged frying time. This occurs because frying food at elevated temperatures facilitates heat and mass transfer between the oil and the meat[39]. Also, the breakdown of the meat's cell walls creates holes that enhance oil absorption. The absorption of oil increases with the elevation of PUFA content in oils[27].

Iodine value

The iodine value determines the unsaturation of an organic component by measuring the quantity of iodine absorbed during a specified time. The presence of double bonds enables the reactions with halogens, which marks iodine value as a significant parameter for analysing the oil's chemical composition and characteristics[20]. The tendency of oil to oxidise when subjected to elevated temperatures during frying increases with a reduction in iodine value, and a decrease in iodine value signifies a decline in the oil's quality, making it unfit for consumption[29]. The iodine value is a crucial parameter in evaluating the oxidative stability of cooking oils, especially post-frying, since it indicates the level of unsaturation in

the oil and helps in determining its oxidation resistance[36].

The iodine value of unfried Cheura oil and the effects of repeated frying with potatoes and chicken meat are illustrated in Fig. 7c. The data indicated that the iodine value of unfried Cheura oil was 22.32 g/100g. In contrast, after frying potatoes in Cheura oil, the iodine value reported after the first frying in P-fry I was 7.39±1.27 g/100g, while after the second frying in P-fry II, the iodine value decreased to -1.11±0.39 g/100g. A reduction in iodine value signifies that lipids have undergone oxidation, resulting in a reduced number of double bonds, indicating an increase in saturation of the oil[9]. Following the third frying in P-fry III, a further reduction in iodine value was seen, measuring -9.70±1.73 g/100g, indicating an increased saturation of the oil. A prior study indicated that frying oil results in a decrease in its unsaturation due to numerous oxidation and thermal degradation processes, which are dependent upon the oil's oxidative stability during frying[36]. When meat is fried in Cheura oil at successive intervals, the initial iodine value recorded in M-fry I was 9.12±1.01 g/100g. Following the second frying in M-fry II, the iodine value decreased to -4.46±0.86 g/100g, indicating a reduction in unsaturation. After the third frying in M-fry III, the iodine value further declined to -11.62±0.001 g/100g, signifying an additional decrease in unsaturation. The comparison of unfried Cheura oil with P-fry and M-fry oils after successive frying showed a significant decrease in iodine value ($P<0.001$). Similarly, a significant decrease in iodine value was observed when comparing P-fry-I with P-fry-II, P-fry-II with P-fry-III, and P-fry-I with P-fry-III ($P<0.001$). Likewise, comparisons of M-fry-I with M-fry-II, M-fry-II with M-fry-III, and M-fry-I with M-fry-III also demonstrated a significant decrease in iodine value ($P<0.001$). For both potato and chicken, there was a decline in iodine value with time, indicating a reduction in the degree of unsaturation of the oil. The decrease of iodine value in potatoes may result from the accumulation of high molecular weight components such as starch. The oxidation rate was more rapid in the oil utilised for frying chicken. Chicken fat has 20–25% palmitic acid, 5–10% stearic acid, 40–45% oleic acid (monounsaturated with one double bond), and 20% linoleic acid (polyunsaturated with two double bonds). During frying, unsaturated fatty acids and lipids melt and drain into the frying medium, where they are swiftly oxidised to produce saturated fatty acids. The saturation level escalates more rapidly in the oil, resulting in a faster decline in iodine value[32].

Saponification value

The saponification value is the quantity of milligrams of KOH necessary to saponify 1 gram of fat under particular conditions, aimed at determining the average molecular weight of the fatty acids involved in this process[19]. A low saponification value signifies longer fatty acid chains and a higher molecular weight, whereas a high saponification value implies triacylglycerols with shorter fatty acid chains[36]. Adulteration of fat or oil with unsaponifiable materials can result in a decrease in saponification value[19].

The saponification value of unfried Cheura oil and the effects of repeated frying with potatoes and chicken meat are illustrated in Fig. 7d. The saponification value of unfried Cheura oil was measured at 12.06 mg KOH/g, which appeared to rise after frying with potatoes, reaching 14.025 mg KOH/g in P-fry I. However, P-fry II and P-fry III exhibited no alteration in the saponification value, remaining at 14.025 mg KOH/g. The oil fried with chicken meat exhibited minimal variation in saponification value, measured at 12.62 mg KOH/g for both M-fry I and M-fry II. However, a small change was noted after the third round in M-fry III, aligning with the saponification value of oil fried with potato, which was 14.025 mg KOH/g. The observed increase in saponification value in the present study may be attributed to the enhanced synthesis of free fatty acids during the frying process with both samples. This may be attributed to the transformation of triglycerides into free fatty acids, leading to an elevated concentration of free fatty acids and ultimately resulting in a rise in the saponification value of the fried oil sample[40]. The comparison of unfried Cheura oil with P-fry oils revealed a highly significant increase in saponification value ($P<0.001$), while comparison with M-fry oils showed a significant increase ($P<0.01$) during the first and second frying rounds. Additionally, during the third frying round, the comparison of unfried oil with M-fry oils demonstrated a highly significant increase ($P<0.001$) in saponification value. Furthermore, comparisons of M-fry-I with M-fry-III and M-fry-II with M-fry-III also indicated a highly significant increase ($P<0.001$) in saponification value.

Ester value

The ester value is defined as the number of milligrams of KOH necessary to hydrolyse the esters contained in one gram of oil sample. The difference between the saponification value and acid value indicates that a higher saponification value correlates with a greater ester value. A high ester value signifies a substantial quantity of esters and the presence of low molecular weight fatty acids in the sample[19].

The ester value of unfried Cheura oil and the effects of repeated frying with potato and chicken meat are illustrated in Fig. 7e. The data obtained revealed that frying potatoes in Cheura oil resulted in an ester value of 13.36±0.74, 13.65±0.43, and 11.27±0.65 mg KOH/g for P-fry-I, P-fry-II, and P-fry-III, respectively, during successive frying rounds. This indicates an increase in ester value compared to the unfried Cheura oil, which had an ester value of 8.85 mg KOH/g, suggesting an increase in ester content and a decrease in fatty acid content[19]. The comparison of unfried Cheura oil with P-fry oils shows a highly significant increase in ester value ($P<0.001$). Similarly, comparisons of M-fry-I and M-fry-III with unfried Cheura oil reveal a significant increase in ester value ($P<0.001$), while the comparison with M-fry-II indicates a significant increase ($P<0.01$) in ester value.

Conclusion

Cheura oil is traditionally used by ethnic communities as a frying medium. However, repeated frying of oils at high temperatures alters their physicochemical properties due to various chemical reactions, making evaluation crucial for quality assessment. A comprehensive analysis of the impact of frying Cheura oil with potato and chicken meat samples was conducted to assess alterations in physicochemical properties. The results demonstrated that repeated frying of potato and meat samples resulted in significant alterations in oil quality due to peroxidation, hydrolysis, and polymerisation. Repeated heating and reuse of oils for frying produce Trans fats and alter fatty acid composition through lipid peroxidation, which marks significant health risks. This research could help the local community in the safe utilisation of Cheura oil by providing safety parameters for culinary practice. The findings highlight the quality and thermal stability of Cheura oil post-frying, providing a safety parameter for community use and emphasising its potential to support rural Himalayan livelihoods.

Cheura oil possesses industrial applicability in the pharmaceutical, cosmeceutical, and equipment manufacturing sectors and serves as a viable source of fatty acids for a range of commercial purposes. Despite its traditional use among rural populations, Cheura oil remains relatively obscure globally. This limited recognition presents a unique opportunity to promote economic development in the western Himalayan regions of India and Nepal while empowering local communities.

Conflict of interest

The authors declare that there is no conflict of interest.

AI use disclosure

ChatGPT (OpenAI) was used solely for language editing and grammar improvement during manuscript drafting. No AI tool was used for data generation, analysis, interpretation, or scientific decision-making.

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