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Research Article

Repetitive Heating Induced Thermal Oxidation of Corn Oil: Impact on Physicochemical Properties, α-Tocopherol, and β-Carotene Depletion

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ABSTRACT

The study aims to analyze the thermal oxidative stability of repeatedly heated corn oil with particular reference to α-tocopherol and β-carotene. The current study evaluated the stability of phytosterol, phenols, α-tocopherol, and β-carotene in corn oil submitted to thermal degradation following repeated heating at 100 and 180°C. The oxygenated samples were repeatedly collected following 5 hours of heat exposure three times a day for three days and stored in amber-colored bottles. Oil samples were subjected to physicochemical parameters along with a quantitative estimation of total phytosterol, total phenols, α-tocopherol, and β-carotene. Thermal degradation at 180°C had elevated density, viscosity, acid value, and peroxide value significantly ($p < 0.05–0.001$), whereas it reduced iodine value and specific gravity. Oxidation of corn oil at 180°C showed 79.18 and 43.75% loss of α-tocopherol and β-carotene content. Heating corn oil three times a day for 5 hours over 3 days results in considerable degradation and darkening of color with a subtle increase in opacity, increased viscosity, density, peroxide value, and acid value, alongside a notable decline in α-tocopherol and β-carotene content. Thermal oxidation led to the formation of oxidized products, resulting in notable alterations in corn oil's physicochemical and phytochemical characteristics, compromising its health benefits.

INTRODUCTION

Corn oil is obtained from corn kernel (*Zea mays*). The quantity of oil in corn varies from 3.5 to 6%. Triacylglycerols in refined corn oil comprise 56 to 59% polyunsaturated fatty acid (PUFA), 24 to 30% monounsaturated fatty acid (MUFA), and saturated fatty acids (13–14%). Linoleic acid is the primary PUFA in corn oil, and it contains a minor amount of linolenic acid. High linoleic acid in corn oil is a dietary necessity for skin integrity, cell membrane functions, and a balanced immune system. Corn oil has considerable ubiquitin and a high quantity of α and β-tocopherols (vitamin E), protecting from oxidative rancidity.[1] The natural antioxidant component, ferulic acid, occurs in an esterified form with ß-sitosterol in corn oil. Corn oil is considered a source of healthy fats when used as part of a balanced diet or in moderation due to its high-calorie content. When heated to high temperatures (above 110°C), the oil undergoes thermal oxidation repeatedly, changing fatty acid isomeric conformation from cis to trans. When reused for cooking or frying, oils undergo oxidation, hydrolysis, and polymerization, forming hydroperoxides and aldehydes.[2] These oxidative products can get absorbed into the fried food, eventually making its way into the gastrointestinal tract after ingestion and enter the systemic circulation.[3]

PUFAs have a more significant potential for deterioration in contrast to their health advantages attributable to high concentrations of double bonds. They produce a wide variety of harmful breakdown products, for instance, aldehydes, ketones, epoxides, hydroxy compounds, etc., and are highly sensitive to oxidative conditions.[4] Cooking

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induces numerous chemical reactions, including thermal oxidation, when oil is subjected to high temperatures, air, and humidity. This results in the breakdown of cooking oil, which produces volatile monomeric and polymeric compounds.[5] Deep frying is the world's most favored and traditional method of preparing food. The oils are used repeatedly for frying to lower food costs. Oil degradation produces free fatty acids, high molecular weight products, and carbonyl compounds depending on increased frying time.[6]

Yuniarti *et al*. studied thermal oxidation-related changes in corn oil at 180°C and continued until 30, 60, 90, and 120 minutes.[7] Thermal oxidation degrades the properties of frying oils. Cooking duration is directly correlated with the deterioration of fatty acid composition and iodine number. Cho *et al*. explored the thermal degradative properties of corn oil heated at ultra-high temperatures in a seasoned laver (300°C for 10 seconds).[8] The oxidative damage induction time of corn oil was 5.3 hours. Substantial changes in the oxidative indices of corn oil, i.e., acid, peroxide, total oxidation, and p-anisidine values, were evident with increased temperature.[9] Cooking corn oil in a conventional oven showed the highest degree of degradation compared to an infrared cooker or an air fryer.[10] Corn oil oxidized at 110°C for 14 hours induces degradation of β-carotene.[11]

Vitamin E analog α-tocopherol is the most efficient antioxidant of the tocopherol isomers. Studies emphasized that α-tocopherol prevents oxidation of dissolved linoleic acid and triglycerides as the temperature increases to 70°C with a broad induction period,[12] whereas, with rising temperature, the oxidation induction period of oils is promptly reduced.[13] The presence of α-tocopherol in corn oil is supposed to provide oxidative stability, but at 180°C heat, α-tocopherol showed a high degradation rate following first-order kinetics.[14] In contrast to the antioxidant effect of α-tocopherol, β-carotene had a pro-oxidant effect in oils.[15] The deterioration rate of β-carotene in oils increases with temperature.[16]

Deep-frying in the presence of air for an extended period is typically involved in the Indian cooking process. In commercial settings, cooking oils are often reused for deep frying multiple times within a day and even on subsequent days. Eating food fried in hot oils on numerous occasions is highly unhealthy. The PUFA in corn oil is primarily linoleic acid, which has low oxidative stability, and its degradation products give the typical deep-fried flavors. To ascertain the stability behavior of corn oil in the conventional cooking process, this study intends to evaluate the physiochemical parameters of oxidized corn oil compared to fresh oil.

The thermal oxidative deterioration was performed at shallow to deep frying temperature ranges (100 and 180°C) repeatedly for 5 hours, three times a day for three consecutive days. None of the earlier studies provide relative concentration level changes in corn oil α-tocopherol and β-carotene subjected to high temperature repeated heating-induced oxidative degradation. Therefore, one-stop estimation is warranted to assess the extent of α-tocopherol and β-carotene degradation and oxidative changes in corn oils. This has not been reported previously, making it a topic of significant interest and primary focus of the project. This article provides insight into the relative stability of phytosterol, phenols, α-tocopherol, and β-carotene in repeatedly heated corn oil.

MATERIALS AND METHODS

Chemicals

Tocopherol and corn oil were purchased from Ozone Pharmaceuticals Ltd., Himachal Pradesh.

Thermal Oxidation of Corn Oil

Fresh corn oil was stored at ambient temperature in a cool, shaded location. Samples of fresh corn oil (200 mL each) were transferred into beakers and individually placed in an electric oven (Model 28, Binder GmbH, Germany) set at 100 and 180°C temperatures. Oil samples were heated for 5 hours, repeated thrice within 24 hours, to hasten lipid oxidation and thermal breakdown. This heating regimen was maintained for an additional 2 days, after which the thermally oxidized oil samples were gathered for subsequent analysis on the third day.

Physicochemical Characterization

The physiochemical characteristics of thermally oxidized corn oil at 100 and 180°C were evaluated compared with regular corn oil samples. Density was determined through mass-to-volume measurements. Refractive index measurements were conducted using a refractometer (Mettler Toledo RM40/RM50, Switzerland). Specific gravity was assessed with a Pycnometer at 25°C following the official AOAC method. Viscosity was measured using a viscometer (Brookfield, DV-E, USA) at a constant speed of 100 rpm with a spindle number S-62, 2 (Brookfield Spindle LV, UK) and reported in centipoise (cP). Acid, peroxide, iodine, and saponification values were determined following standard procedures outlined in AOAC and AOCS guidelines.[17,18]

Phytochemical Characterization

Phytosterols

Phytosterol content was analyzed following the procedure outlined by Cercaci *et al*. with minor adjustments.[19] Oil sample (1-mL) was combined with potassium hydroxide in ethanol (4 mL; 2 mol/L) and subjected to shaking in a heated water bath (90°C) for 1-hour to facilitate saponification. Following cooling, n-hexane (5 mL) and deionized water (1-mL) were added. The resulting solution was vigorously shaken to extract an unsaponifiable matter.

The supernatant was extracted thrice with hexane and combined using the same procedure.

Total phenols

The liquid-liquid extraction method was employed to separate the phenolic fraction of the oil samples through phase-wise vortexing and phase separation. In the initial phase, the oil sample was treated with a methanolic hexane solution (1:1), followed by hexane-ethyl acetate (85:15), and finally eluted with methanol and vacuum evaporated at 35°C. Total phenolic concentration was estimated by spectrophotometric scanning at 765 nm following the Folin-Ciocalteu method and expressed as GAE mg (gallic acid equivalents) per 100 gm of oil.[20]

β-carotene

The procedure outlined by Gimeno *et al*. was adhered to with some adjustments for the isolation and quantification of β-carotene.[21] After extracting unsaponifiable matter, the organic phase was evaporated using a rotary evaporator (Rotavapor R-300, Fisher Scientific, USA) at 40°C. The resulting residue was evaporated under a nitrogen stream and then reconstituted in methanol. Quantification was promptly carried out to prevent β-carotene oxidative decomposition. The β-carotene compositions in oil samples were estimated using a spectrophotometer (SL160, India). The β-carotene concentration oil samples were calculated as micrograms per gram.[22]

α-Tocopherol

For the qualitative estimation of tocopherols, a high-performance liquid chromatography (HPLC; Agilent Technologies 1260, USA) system was utilized, comprising a 515 pump, a UV-visible detector, a Thermo C 18 (250 × 4.6 mm) 5 µm column, and Data Ace software. The mobile phase was water-methanol-acetonitrile (90:5:5 v/v) at 1-mL/min flow rate. The phenolic residue was solubilized in methanol and water (1:1), sonicated for 25 minutes, and filtered (0.45 µm). This stock solution was appropriately diluted with methanol to get a 10 µg/mL concentration. A sample volume of 20 µL was injected, and tocopherol retention time was detected at 290 nm.[23] Tocopherol concentration was expressed in µg/gm of oil.

STATISTICAL ANALYSIS

All data were presented as Mean ± SEM. Student's t-test was performed to test statistical significance. Variance (ANOVA) was analyzed to assess group differences and, subsequently, Tukey's multiple comparison test. A *p-value* of $p < 0.05$ was regarded as statistically significant.

RESULTS

Physicochemical Properties of Thermally Oxidized Corn Oil

Thermal oxidation of corn oil with periodically repeated heating for three days accelerated thermal degradation and changed the oil from a golden yellow color to light amber with low rancidity (Fig. 1). Thermal oxidation at 100°C impacted the chemical composition of the corn oil, with a significant increase in density and viscosity ($p < 0.05$) and reduction in specific gravity ($p < 0.01$). Repeated heating at 100°C does not have any effect on the refractive index. The 180°C thermal oxidation process has a potential impact resulting in very significant ($p < 0.01–0.001$) increase in density, viscosity, and refractive index. Repeated high temperature has decreased specific gravity significantly ($p < 0.001$) (Table 1).

Thermal degradation at 100 and 180°C has significantly ($p < 0.05–0.001$) increased acid value and peroxide value, while iodine value ($p < 0.01$) was decreased. The increase in saponification value was significant only following degradation at 180°C (Table 2).

Phytochemical Properties of Thermally Oxidized Corn Oil

Thermal degradation of corn oil at 100 and 180°C had significantly ($p < 0.05–0.001$) decreased phytosterol and total phenols content. Oxidation of corn oil at 100 and 180°C exhibited, respectively 10.41, and 43.75% loss of

Fig. 1: Normal and thermally oxidized corn oil at 100 and 180°C for 3 days

Table 1: Physical characteristics of thermally oxidized corn oil

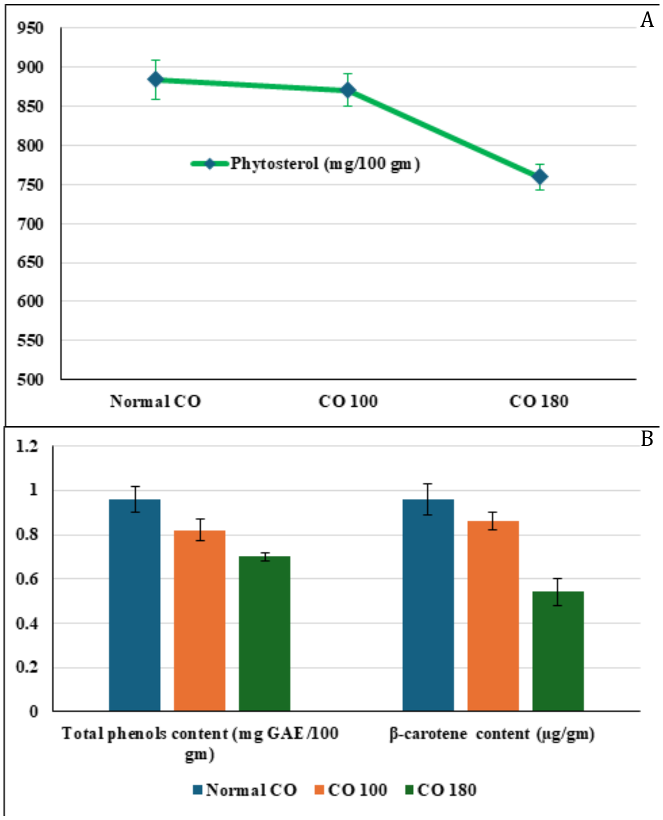
Categories	*Density (gm/mL)*	*Specific gravity*	*Refractive index*	*Viscosity (centipoise)*
Normal CO	0.915 ± 0.003	0.916 ± 0.003	1.527 ± 0.005	12.51 ± 0.89
CO 100	0.972 ± 0.009*	0.876 ± 0.034**	1.533 ± 0.002 ns	14.92 ± 1.26*
CO 180	0.985 ± 0.005**	0.852 ± 0.002***	1.580 ± 0.004**	18.65 ± 1.50***

All the values are Mean ± SEM of three values (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ns = not significant when compared to the respective normal CO group. CO = Corn oil, CO 100 = Corn oil oxidized at 100°C, and CO 180 = Corn oil oxidized at 180°C.

Table 2: Chemical characteristics of thermally oxidized corn oil

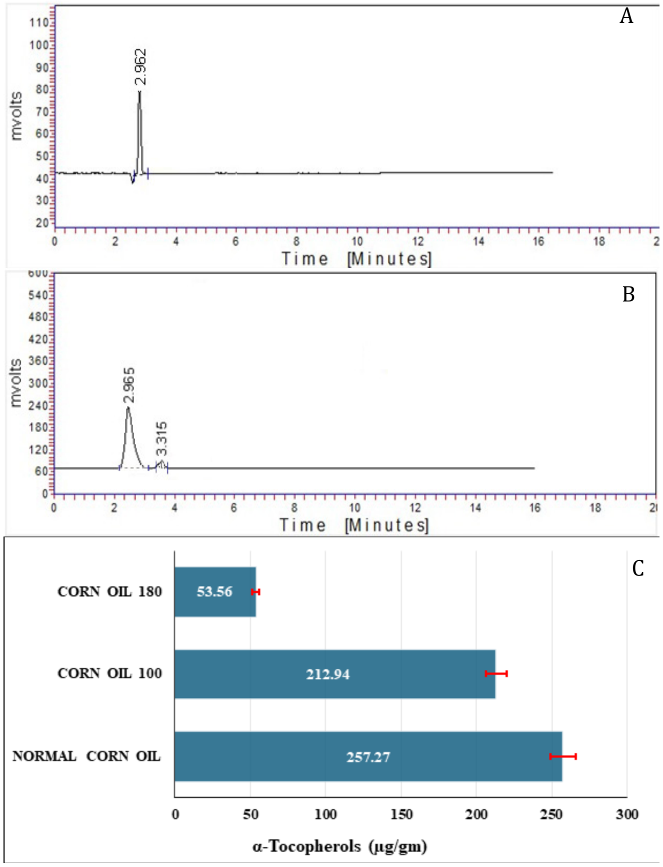
Categories	*Acid value (mg KOH/gm)*	*Saponification value (mg KOH/gm)*	*Peroxide value (meq 02/ kg)*	*Iodine value (gm I2/100 gm)*
Normal CO	3.51 ± 0.03	191.59 ± 1.47	.0.156 ± 0.004	128.23 ± 2.20
CO 100	4.32 ± 0.06***	192.56 ± 1.36ns	0.746 ± 0.005**	122.52 ± 1.46*
CO 180	6.61 ± 0.04***	194.51 ± 2.15*	1.934 ± 0.008***	121.14 ± 1.38**

All the values are Mean ± SEM of three values (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ns = not significant when compared to the respective normal CO group. CO = Corn oil, CO 100 = Corn oil oxidized at 100°C, and CO 180 = Corn oil oxidized at 180°C.



Phytosterol (A), total phenols and β-carotene content (B). All the values are Mean ± SEM of three values (n = 3). *$p < 0.05$, ***$p < 0.001$, and ns = not significant when compared to the respective normal CO group. CO = Corn oil, CO 100 = Corn oil oxidized at 100°C, and CO 180 = Corn oil oxidized at 180°C.

Fig. 2: Phytochemical characteristics of thermally oxidized corn oil



HPLC retention time of α-tocopherols in normal corn oil (A) and thermally oxidized corn oil (B).

Fig. 3: α-Tocopherols level in the normal and thermally oxidized corn oil

β-carotene content (Fig. 2). HPLC chromatogram showed the qualitative presence of α-tocopherol in the normal and thermally oxidized corn oil samples. α-Tocopherol content was reduced by 17.24 and 79.18%, respectively, following the thermal oxidation of corn oil at 100 and 180°C (Fig. 3).

DISCUSSION

High-temperature repeated heating induces oxidative degradation. Therefore, a one-stop estimation has been attempted to quantify α-tocopherol and β-carotene degradation and oxidative changes in corn oils under high-temperature repeated heating. This subject matter is of significant curiosity, making it the foremost focus of this study. This article provides insight into the relative stability of phytosterol, phenols, α-tocopherol, and β-carotene in repeatedly heated corn oil.

Heating corn oil for 5 hours thrice a day for three days darkens color from light yellow to amber. Darkening of color arises from the development of pigments during oxidation and thermal breakdown of fatty acids. Additionally, traces of carotenoids may contribute to this darkening.[24] The mild oxidative rancidity observed in the heated corn oil is due to the oxidation of double bonds in fatty acids, forming aldehydes, ketones, and lower molecular weight acids. The presence of oxygen, light, moisture, and specific metal catalysts influences the extent of oxidative rancidity. The slight rancidity observed in 180°C heated corn oil could be attributed to the controlled oven heating of oil samples under the experimental conditions. Heating was conducted in an oven without light exposure and food ingredients containing moisture. The primary cause of oxidative rancidity is the oxidation of oleic acid in corn oil. Heating vegetable oil at 105°C for 12 hours resulted in a linear darkening of color and a reduction in α-tocopherol levels. Interestingly, phytosterol did not influence color reversion.[25]

Thermal oxidation of corn oil following repeated heating at 180°C induced conversion in chemical composition of corn oil, resulting in a significant elevation of density and viscosity with a lowering of specific gravity. Oil viscosity is influenced by factors like chain length and/or saturation/unsaturation level of triglycerides. Alterations in the organization of fatty acids on the glycerol backbone of triglyceride molecules induce change in viscosity. According to Kim *et al*.[26] repeated heating-induced hydrogenation of corn oil at 180°C led to a substantial increase in viscosity and density, indicating heightened saturation and polymerization, alongside a decrease in unsaturation. Corn oil subjected to boiling and several frying cycles with potato pieces exhibited an increase in viscosity and peroxide value, consistent with the findings of the study. However, there was a decrease in density in contrast to the observation of the current study.[27]

Thermal degradation caused a significant reduction in corn oil specific gravity due to the degradation of its constituents and a subsequent lowing of density. Similarly, repetitive heating of palm oil at 180°C during food frying decreased specific gravity, as Idun-Acquah *et al*. reported.[28]

Thermal oxidation resulted in a notable increase of peroxide, acid, and saponification values, alongside a decrement of iodine value. Oil peroxide value reflects the amount of hydroperoxides generated during oxidation, while the acid value indicates the level of free fatty acids.[29]

The observed changes in peroxide value in corn oil suggest substantial oxidation after being subjected to heating for 5 hours, three times a day, over three days. The decline in the iodine value of heated oil signifies an accelerated oxidation rate. The iodine value indicates the extent of unsaturation or double bonds in fatty acids of oil, and the decreased value signifies the higher number of double bond oxidation in heated oil. A significant alteration in iodine values indicates excessive oil deterioration.[30]

While saponification and acid values are not typically utilized for measuring oil oxidation, they offer insightful details about the oxidation state of oil. The average molecular mass of oil fatty acids is indicated by saponification value, which is inversely correlated with the molecular weight. The tertiary oxidation-related acidic product formation correlates with the relative increment of acid value following heating.[31] Oxidation leads to the breakdown of fatty acid chains, thereby elevating the saponification value. Congruous with the findings, at 180°C, heating of corn oil for 120 minutes showed a time-proportional increase in free fatty acid and conjugated diene content along with peroxide and thiobarbituric acid value. Thermal oxidation causes the formation of trans fatty acids from their natural cis-isomer following exposure to high temperature with significant conversion of physicochemical properties.[7]

The oxidation induction time is 5.3 hours for regular corn oil, 3.3 hours for heated corn oil, and 4.1 hours for heated corn oil applied to seasoned laver. The acid and peroxide values increased in all the heated conditions, with a minor decrease in radical scavenging activities.[8]

The heightened saponification values observed after thermal degradation of different vegetable oils at 180°C suggest a greater degree of oxidation in the oil.[32] The increased peroxide, saponification, and acid value of corn oil post-frying align with findings from Alajtal *et al*.[33] However, specifics regarding frying temperature and duration are not provided. Saeeda and Naz evaluated how conventional heating, compared to microwave heating, affected the oxidative characteristics of corn oil.[9] The findings demonstrated that with rise in temperature (30–70°C), substantial changes in oxidative indices like acid, peroxide, and p-anisidine values occur. The development of secondary oxidative components during the chemical decomposition of the oil fatty acids was more profound during microwave heating.

Ostlund *et al*. revealed that campesterol, campestanol, sitosterol, and stigmasterol comprised 0.773% of the phytosterols found in corn oil.[34] Interestingly, in this study, regular corn oil exhibited a higher phytosterol content of 0.884%, and phenolic compounds possessing robust antioxidant and anti-polymerization properties during high-temperature frying processes. This may be attributed to phenolic compounds' lower volatility, improved solubility at high temperatures, and the formation of secondary products with diverse antioxidant activities.[35] The elevated phytosterol content observed in this study underscores the significant variability in phytosterol content among oils due to variety, growing geography, oil production process (pressing, refining), storage and saponification conditions.[36]

The thermal degradation of corn oil at 180°C had significantly decreased phytosterol and total phenols content along with a 79.18% loss of α-tocopherol content. The degradation of tocopherols during oil frying is a well-recognized process primarily influenced by temperature, duration of cooking, and the variety of oil utilized.[37] Elevated temperatures and prolonged heating durations accelerate the degradation of tocopherols in oil. Tocopherol degradation rate in oil is influenced by the degree of unsaturation as well as proportions of α-, β-, γ-, and δ-tocopherols. Although α-tocopherol possesses the maximum biological properties, it degrades the fastest during frying.[38] For instance, rice bran oil α-tocopherol exhibited a reduction of 28.65% at 100°C and complete degradation (100%) after continuous heating for 240 hours at 180°C.[37] However, the relatively slower decomposition of tocopherols in corn oil can be attributed to phenolic acids and various antioxidant compounds. Additionally, highly unsaturated compounds and PUFAs actively participate in the oxidation cascade, which can slow down the degradation of tocopherols.[39]

Oxidation of corn oil at 100 and 180°C resulted in, respectively, loss of 10.41 and 43.75% of β-carotene. The β-carotene degradation in corn oil initiates upon prolonged heating above 110°C, leading to various oxidative products of triacylglycerols, such as epidioxy and hydroxy bis-hydroperoxides. Zeb and Murkovic characterized β-carotene and other oxidative degradation products of corn oil cooked at 110°C for 1 to 14 hours.[11] They observed a considerable degradation of β-carotene following accelerated thermal degradation of corn oil. Major oxidative degradants of β-carotene were 8′-apo-β, 6′-apo-β, and 5,6-epoxy-8′-apo-β-carotenal; β-carotene-2,2′-dione; 13-Z-5,6,5′,6′-diepoxy, all-E-5,8-epoxy, all-E-5,6-epoxy-, and 15-Z-5,6-epoxy-β-carotene. β-Carotene promotes the oxidative breakdown of triacylglycerols in corn oil, particularly with longer exposure times, leading to the prominent epidioxy and hydroxy bis-hydroperoxides formation. Further degradation components of triacylglycerols are mono-epoxides and mono- and epoxy hydroperoxides.

The research outcome provides a singular standpoint on thermal oxidative alterations of corn oil following repeated use for deep frying. Subjecting corn oil to repeated heating cycles, especially at 180°C, thrice daily for 5 hours over three days caused considerable degradation. This degradation was characterized by increased viscosity, density, peroxide value, and acid value, alongside a notable decline in α-tocopherol and β-carotene content of oxidized corn oil. Lipid oxidation and hydrolytic reactions occur during the frying activities, causing degradation of oil quality.[40,41] Triacylglycerol undergoes primary oxidative changes, producing hydroperoxides that break down into low molecular weight components like free fatty acids, alcohols, aldehydes, and ketones. Repeated heating of frying oils at high temperatures induces extensive decomposition and complicated chemical transformations. Exposure to moist air and water accelerates deterioration, generating large amounts of polar compounds evidenced by elevation of viscosity and density.[42]

CONCLUSION

Corn oil has a high content of PUFA, linolenic acid, ubiquitin, α and β-tocopherols and ferulic acid is responsible for numerous health benefits. Corn oil is a source of healthy fats, provided it is consumed as part of a balanced diet and in moderation, owing to its high-calorie content. The study outcome indicates that the health-protective properties of corn oil can diminish gradually with repeated heating at high temperatures. The thermal oxidation induced by high-temperature cycles led to the formation of oxidized products, resulting in notable alterations in physicochemical and phytochemical characteristics. This research delves into the thermal oxidative degradation pattern of corn oil following multiple time heating at usual deep frying temperatures. However, it does not investigate the interaction with moisture, which could be explored by frying corn oil in the presence of food materials. Additional studies are underway to evaluate the impact of thermally oxidative corn oil *in-vivo.*

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