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Effect of thermal processing on the quality parameters of ghee incorporated with curry leaf (*Murraya koeneigii*) extract#

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Abstract

This study examined the effect of adding antioxidants, namely curry leaf extract (CLE) and butylated hydroxyanisole (BHA), on the physicochemical properties of ghee during deep frying. The results showed that the addition of CLE to ghee resulted in significant changes in the L, a*, and b* values, likely due to the extraction of chlorophyll molecules during CLE preparation. Deep frying caused a decrease in L* values and an increase in a* and b* values of ghee, as well as an increase in the redness and yellowness indices, with control ghee experiencing the most significant changes. The viscosity of ghee increased during frying, and the increase was less in ghee with added antioxidants. Antioxidant-added ghee showed the least increase in total polar compounds (TPC) and thiobarbituric acid reactive substances (TBARS) during frying, which are indicators of oil degradation. CLE and BHA were found to inhibit the production of Cholesterol Oxidation Products (COPs) in ghee during deep frying for up to 45 minutes. Control ghee showed the presence of both COPs (5α, 6α-epoxide and 7β-hydroxy cholesterol) after 45 minutes of frying, whereas ghee with added CLE and BHA had only 5α, 6α-epoxide after 45 minutes of frying.*

Keywords: *Ghee, curry leaves, cholesterol oxidation products, total polar compounds*

Fast food has recently gained popularity among young people as part of their diet. Food is prepared in restaurants and at home using a variety of techniques, but frying is the most well-liked, preferred, and frequently used method because it produces food that is both delicious and

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aesthetically pleasing. Additionally, it is the fastest, best, and easiest technique of cooking because food can be fried by being immersed for a short period of time in heated oil. Cooking food in oil at a temperature of 150 to 190 °C is known as deep fat frying (Aydınkaptan and Mazı, 2017). When prepared properly, fried food has an appealing flavour, a golden-brown outside and a crispy interior.

Ghee has several advantages as a frying oil. It has a rich, nutty flavour that can enhance the taste of the food. Ghee is also a good source of healthy fats and essential vitamins like A, D, E, and K. It has a longer shelf life compared to other oils and can be stored at room temperature without getting spoiled. The development of oxidation products is significantly impacted by the high temperature used in frying, which favours the production of oxidised and non-oxidized dimers and polymers. Like triglycerides, free fatty acids, a byproduct of hydrolysis, are more susceptible to heat and oxidative change (Angelo and Jorge, 2008). When food is fried at a high temperature in the presence of oxygen and moisture the ghee or oil undergoes significant changes including oxidation, polymerization, cyclization, and hydrolysis. Rai and Narayanan (1984) have investigated the development of carbonyl compounds in ghee and refined ground nut oil during intermittent frying. Continuous monitoring of frying oil quality is crucial due to its inconsistent nature and tendency to often degrade exceeding the specified limits, rendering it unsuitable for further use (Croon *et al*., 1986; Navas *et al*., 2007). The potential health hazards associated with consuming heated and/or oxidised lipids, such as the likelihood of developing atherosclerosis, as well as their mutagenic and carcinogenic effects, have been studied (Kubow, 1990).

Recent studies on ghee have generated interest in employing natural antioxidants as opposed to the legal synthetic BHA (Pawar *et al*., 2012; Patel *et al*., 2013). Since BHA does not function as a potential antioxidant at higher temperatures, it has already been documented that oils and fats containing it frequently deteriorate quickly when being used for frying (Tsaknis *et al*., 2002). Patel *et al.* (2013) had demonstrated that when ghee is deep-fried, BHA is less stable than coriander extract. Curry leaves (*Murraya koenigii*) are most widely used in southern and western parts of India for cooking as seasonings. The plant is highly valued for its leaves which are used for flavouring and spicing of food. Mahanimbine and koenigine, two recently discovered carbozole alkaloids present in curry leaves, exhibit stronger antioxidant activity (Igara *et al*., 2016). In solid-liquid batch extraction, more recovery of total polyphenols was obtained for 50% (v/v) aqueous methanol and at 333 K temperature. The total polyphenol obtained at optimum conditions was 79.34 mg GAE/L (Patil *et al*., 2021). Thus, the current study's main objective was to investigate how the quality characteristics of ghee with CLE were affected by thermal processing.

Materials and methods

Preparation of ghee samples

The oil soluble curry leaves extract (CLE) was obtained from Citspray aroma Sciences, Nagpur, Maharashtra. A 40–42% fat cream was collected, cooled to 8–10°C and aged overnight at same temperature. The aged cream was churned into butter at 10°C, which was then clarified at 110 ± 1°C and then filtered through cheesecloth (Rahila *et al*., 2018). After that, the ghee was cooled to 60°C and the CLE at 1% level was added based on sensory evaluation. No CLE was added in the control ghee. In this study, ghee with 0.02 percent BHA was also employed as a comparison.

Analysis of sample

A commercial gulab jamun ready mix was used to make the dough, and 100 balls weighing 6-7 grams each were fried in 1500 grams of ghee at 180°C for 60 minutes in a stainless-steel deep fryer (AGARO-33390 Marvel 1700-Watt Deep Fryer). Physico-chemical parameters like colour (IS. 3508, 1966), viscosity, TBA value, total polar compounds and cholesterol oxidation products of ghee samples were analysed for each 15 min interval.

Colour

Colour of the ghee samples was measured by reflectance spectroscopy technique as per IS. 3508, 1966 employing reflectance meter, colour flex with geometry of diffuse/8° (sphere-8 mm view) and an illuminant of D65/10°. Before the test, the instrument was calibrated with standard black glass and white tile as specified by the manufacturer. The light source was dual beam xenon flash lamp. Data were received from the software in terms of L* [Lightness, ranges 0 (black) to 100 (White)], a* [Redness, ranges from +60 (red) to -60 (green)] and b* [Yellowness, ranges from +60 (yellow) to -60 (blue)] in values of the international colour system.

Viscosity

To evaluate the viscosity of ghee samples, a Brookfield viscometer with a jacketed small sample adaptor and an S18 spindle was utilised (Sahasrabudhe *et al*., 2017). A sample cup was filled with five millilitres of the sample, which was then held at a temperature of 31°C for 60 seconds before measurement. Readings were made at 50 revolutions per minute, and the result was given in centipoise (cP).

Thiobarbituric acid value

The Patton and Kurtz (1951) method was used to measure the thiobarbituric acid (TBA) levels. One millilitre of trichloroacetic acid (35%) and two millilitres of TBA reagent (0.36 grammes of TBA (2-Thiobarbituric acid) and 0.1 grammes of anhydrous sodium sulphate in 100 millilitres of distilled water) were added to approximately 0.1 grammes of molten ghee samples that had been precisely weighed into a centrifuge tube. The contents were vigorously shaken. For 15 minutes, the tubes were incubated in a bath of boiling water. The mixture was cooled, combined with 1 mL of glacial acetic acid and 2 mL of chloroform, then centrifuged at 500 g for 5 min to separate the contents into two layers that were easily distinguishable. Using a spectrophotometer, the optical density (OD) of the supernatant was determined at 532 nm. The results were presented as the number of TBA reactive substance (TBARS) per 0.1 g of ghee. At the same time, blank was made without ghee.

Total Polar Compounds (TESTO 270)

Total polar compounds are measured by a handy measuring instrument called testo 270 cooking oil tester. The oil samples were filtered in order to remove residues of the product being deep fried and parts of the degradation products of the fat and water bonded to these from the fat. Then the oil was taken in a 50 mL beaker and temperature adjusted to 40°C using a water bath. The sensor of testo 270 was then immersed in to the sample and readings were recorded.

Cholesterol oxidation products (COPs)

Identification of COPs in ghee samples was carried out using the method of Nath *et al*. (1996). The method is described as follows: For estimation of COPS, first the ghee samples were saponified by cold saponification method. The unsaponifiable matter extracted was applied on TLC plate for separation and identification of COPs.

Ghee sample (2 g) was accurately weighed in a 250 mL round bottom flask. To this, 50 mL of 0.5 N ethanolic potassium hydroxide solution was added. The contents were vigorously shaken for a few min and saponified for 2 h under nitrogen. The saponified material was transferred to a 250 mL separating funnel, the flask was rinsed with 25 mL distilled water and added to funnel. The unsaponifiable matter (USM) was extracted thrice with 25 mL portions of diethyl ether. Pooled ether extract was washed thrice with 25 mL portions of 0.5 N aqueous potassium hydroxide, followed by water until it became free from alkali. Ether extract was filtered through Whatman No.1 filter paper over anhydrous sodium sulphate. Ether was evaporated under reduced pressure and the USM was collected with 5 mL chloroform.

The USM obtained was applied in small volume (20 µL) on Silica gel 60 F254 TLC plate (Aluminium sheets, 20X20 cm). The plates were then developed in developing solvent (heptane/ethyl acetate, 1:1 v/v). The spots were visualized by spraying the plates with 10% copper sulphate in 8% orthophosphoric acid,

followed by heating at 110^{0}C in a hot air oven for 8 min. The separated COPs and cholesterol were identified by comparison with standard COPs and cholesterol was separated under same chromatographic conditions

Statistical analysis

The data were presented as mean values and standard errors. The analysis of variance (ANOVA) was used in each analysis to statistically analyse the data from three replications with a significance level of $p < 0.05$, and the Tukey's multiple comparison test was used to compare the treatment means in SPSS (IBM Corporation, Armonk, NY).

Results and discussion

Colour

The colour values of ghee samples were measured using a Hunter lab colorimeter, and the results were reported as L* (lightness), a* (redness), and b* (yellowness). There was an initial significant variation in L*, a*, and b* values ($p<0.05$) of CLE added ghee as compared to other samples. This is due to chlorophyll molecules may also have been extracted while preparing CLE. Adding this extract to the ghee may lead to changes in the original colour of the ghee. Moreover, this colour change may be detected only during instrumental analysis and not perceived at subjective analysis of colour. Ahmad (2014) claims that the rapid shift in oil colour during frying—from light yellow to orange/brown—is caused by the oxidation reactions that result in coloured compounds. According to Tarmizi *et al.* (2013) frying causes the oil to darken relative to fresh oil by increasing the a* and b* values and decreasing the L* value. The test results were consistent with earlier studies; Table 1 shows a drop in L* values and an increase in a* and b* values of ghee after frying. The control ghee's percent lightness significantly decreased ($p<0.05$) after 60 minutes of frying, falling from 56.28±0.05 to 38.6±0.13. Ghee added with antioxidants, on the other hand, showed a lesser decrease in per cent lightness (from 56.31±0.05 to 45.16±0.12 and 60.12±0.07 to 52.64±0.05 for ghee added with BHA and CLE, respectively).

The redness index of ghee increased significantly ($p<0.05$) during deep frying. In control ghee this value increased rapidly, reaching 10.74±0.19 from 1.45±0.01 at 60 minutes of frying. Ghee added with other BHA and CLE also showed an increase in redness index during deep frying but with a slow rate of increase as compared to control ghee (from 1.48±0.12 to 6.86±0.07 and 0.34±0.06 to 5.56±0 for ghee added with BHA and CLE respectively). Similarly, the yellowness index

Table 1. Lightness (L*) index (%), Redness (a*) index (%) and Yellowness (b*) index (%) of ghee added with antioxidants during deep frying (Mean ± SE, n=3)

Ghee added with	Colour	Frying time (min)				
		0	15	30	45	60
Control	L*	56.28±0.05^{Aa}	50.56±0.53^{Ab}	40.45±0.17^{Ac}	40.12±0.30^{Ac}	38.60±0.13^{Ad}
	a*	1.45±0.01^{Aa}	5.88±0.13^{Ab}	10.08±0.22^{ac}	10.18±0.40^{Ac}	10.74±0.19^{Ac}
	b*	19.85±0.17^{Aa}	36.07±0.77^{Ab}	45.88±0.40^{Ac}	51.44±0.73^{Ad}	57.34±0.87^{Ae}
BHA	L*	56.31±0.05^{Aa}	54.29±0.67^{Bb}	53.89±0.23^{Bb}	49.08±0.08^{Bc}	45.16±0.12^{Bd}
	a*	1.48±0.12^{Aa}	3.47±0.14^{Bb}	3.58±0.29^{Bb}	5.43±0.19^{Bc}	6.86±0.07^{Bd}
	b*	19.90±0.24^{Aa}	35.18±0.34^{Ab}	39.67±0.44^{Bc}	39.72±0.08^{Bc}	40.28±0.33^{Bc}
CLE	L*	60.12±0.07^{Ba}	56.66±0.26^{Cb}	55.95±0.49^{Cb}	52.73±0.13^{Cc}	52.64±0.05^{Cc}
	a*	0.34±0.06^{Ba}	2.55±0.23^{Cb}	3.04±0.10^{Bb}	3.23±0.33^{Cb}	5.56±0.06^{Cc}
	b*	36.42±0.42^{Ba}	37.95±0.09^{Bb}	40.63±0.32^{Bc}	44.64±0.15^{Cd}	47.03±0.48^{Ce}

a, b: means within columns with different lowercase superscripts are significantly different ($p<0.05$) from each other

A, B: means within rows with different uppercase superscripts are significantly different ($p<0.05$) from each other

of ghee increased significantly ($p<0.05$) during frying. For control ghee, it increased rapidly and reached to 57.34±0.87 from 19.85±0.17 at 60 minutes of frying. Ghee added with BHA and CLE showed a slower rate of increase in yellowness index (from 19.9±0.24 to 40.28±0.33 and 36.42±0.42 to 47.03±0.48 for ghee added with BHA and CLE respectively)

Viscosity

Table 2 shows the influence of BHA and CLE addition on the viscosity (cp) of ghee during deep frying. The viscosity of the samples increased while frying, as may be seen from the data in the table. After 60 minutes of frying, the viscosity of the control ghee increased significantly from 36±.0.0 to 43.16±0.16 cp. However, the increase in viscosity is less in ghee with BHA and CLE (from 36.1±0.16 to 41.5±0.28 and 35.8±0.16 to 39.9±0.20, respectively).

Nayak *et al*. (2016) reviewed that higher temperatures and the number of frying cycles have a significant impact on the viscosity of ghee. It could be the result of the formation of oxidative and polymeric chemicals during the frying process. According to Sharoba and Ramadan (2012), frying time has an impact on the increase in viscosity of oils during frying. The increase in viscosity suggests that there has been a significant structural shift. This has been linked to the formation of polymers, which are the degradation products with the highest molecular weight compounds and their level increased as the frying time was increased.

Table 2. Viscosity of ghee added with antioxidants during deep frying (Mean ± SE, n=3)

Viscosity of ghee at 31°C (cP)			
Frying interval (min)	Ghee added with		
	Control	BHA	CLE
0	$36±0.0^{Aa}$	$36.1±0.16^{Aa}$	$35.8±0.16^{Aa}$
15	$36.5±0.5^{Aa}$	$37.16±0.44^{Ba}$	$36.83±0.60^{Ba}$
30	$38.5±0.29^{Ba}$	$38.23±0.23^{Ca}$	$37.66±0.33^{BCa}$
45	$40.06±0.29^{Ca}$	$40.16±0.33^{Da}$	$38.5±0.28^{Cb}$
60	$43.16±0.16^{Da}$	$41.5±0.28^{Eb}$	$39.9±0.20^{Dc}$

a, b: means within columns with different lowercase superscripts are significantly different ($p<0.05$) from each other

A, B: means within rows with different uppercase superscripts are significantly different ($p<0.05$) from each other

Table 3. Total Polar Compounds of ghee added with antioxidants (Mean ± SE, n=3)

Total Polar Materials (%)			
Frying interval (min)	Ghee added with		
	Control	BHA	CLE
0	$19.13±0.06^{Aa}$	$19.1±0.05^{Aa}$	$19.08±0.06^{Aa}$
15	$20.16±0.16^{Ba}$	$19.9±0.1^{Ba}$	$19.96±0.11^{Ba}$
30	$21.23±0.2^{Ca}$	$21.13±0.21^{Ca}$	$20.95±0.39^{Ca}$
45	$22.36±0.03^{Da}$	$21.93±0.03^{Dab}$	$21.4±0.13^{Db}$
60	$23.2±0.2^{Ea}$	$22.73±0.17^{Eab}$	$22.15±0.2^{Eb}$

a, b: means within columns with different lowercase superscripts are significantly different ($p<0.05$) from each other

A, B: means within rows with different uppercase superscripts are significantly different ($p<0.05$) from each other

Total polar compounds

Total polar compounds were quantified using the TESTO 270, a hand-held device for cooking oil testing. The dielectric constant of oil samples is significantly connected with the concentration of TPC and the Testo 270 cooking oil tester monitors variations in the dielectric constant of oil samples. Table 5 shows the effect of added BHA and CLE to ghee during deep frying on the production of TPC. There was no significant difference between control and antioxidant added ghee samples at initial TPC levels (about 19.1%) of ghee samples. At the end of frying, there was a substantial increase in TPC values of all samples and antioxidant added ghee showed the least rate of increase. The values were 23.2±0.2, 22.73±0.17 and 22.15±0.2 for ghee samples control, BHA added ghee, and CLE added ghee, respectively. FSSAI had fixed a limit for total polar compounds at 25 per cent beyond which the vegetable oil should not be used. When polar compounds reach 24 per cent, the oil should be rejected and replaced with new oil (Ahmad, 2014). TPC of sunflower oil increased from 6.2 per cent in the initial sunflower oil to 18.7 per cent after 15 frying cycles (Garrido-Polonio *et al.,* 1994*).*

TBA Value

The impact of antioxidants (CLE and BHA) on the generation of TBARS during ghee frying was studied, and the findings are shown in Table 4. It is evident from the data in the table that CLE as well as BHA were significantly effective in limiting the rise in TBA values compared to the control ghee sample. All samples demonstrated a considerable increase in TBA value after 15 minutes of frying, with the ghee with CLE having the least rate of increase (0.27±0.001, 0.227±0.009, and 0.151±0.004 for ghee samples control, ghee with BHA, and ghee with CLE, respectively). At the end of frying, the TBARS of ghee samples had increased to 0.382±0.003, 0.299±0.002, and 0.242±0.002 for control, BHA added ghee, and CLE added ghee, respectively. After the first and second frying cycles, ghee with added clove, green tea and BHA had significantly lower TBA values than those containing coriander, vidarikand, ashwagandha and shatavari ($p < 0.05$). At the end of the fourth frying cycle, it was shown that ghee containing clove (0.20±0.05), green tea (0.21±0.01), and coriander (0.21±0.04) had considerably lower TBA values ($p < 0.05$), than ghee containing BHA (0.27±0.06) (Patel *et al.,* 2014).

Cholesterol oxidation products (COPs)

The presence of cholesterol oxidation products (COPs) in foods has evoked much interest due to health concerns. The effect of antioxidants (CLE and BHA) added on the development of COP in ghee during deep-frying was detected by TLC and the chromatograms are presented in Fig. 1.

Table 4. Thiobarbituric acid value of ghee added with antioxidants during deep frying

Thiobarbituric acid value (TBARS/0.1 g fat)			
Frying interval (min)	Ghee added with		
	Control	BHA	CLE
0	0.028. ±0.001^{Aa}	0.025±0.000^{Aa}	0.026±0.000^{Aa}
15	0.270±0.001^{Ba}	0.227±0.009^{Bb}	0.151±0.004^{Bc}
30	0.285±0.00^{Ca}	0.254±0.005^{Cb}	0.179±0.004^{Cc}
45	0.350±0.006^{Da}	0.262±0.001^{Db}	0.202±0.003^{Dc}
60	0.382±0.003^{Ea}	0.299±0.002^{Eb}	0.242±0.002^{Ec}

Mean ± SE, n=3.

a, b: means within columns with different lowercase superscripts are significantly different ($p<0.05$) from each other

A, B: means within rows with different uppercase superscripts are significantly different ($p<0.05$) from each other

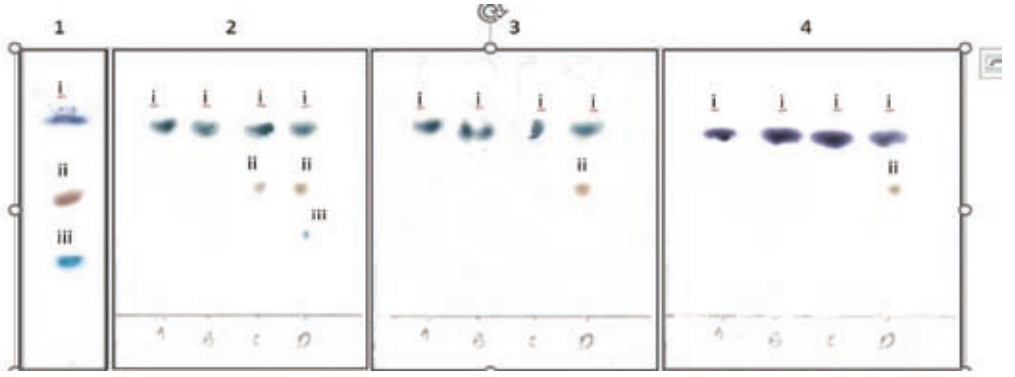

Fig. 1. TLC chromatogram of unsaponifiable matter isolated from ghee used for deep frying 1 = standards; 2 = control ghee used for deep frying for 0, 30, 45, and 60 min; 3 = ghee added with BHA used for deep frying for 0, 30, 45, and 60 min; and 4 = ghee added with CLE used for deep frying for 0, 30, 45, and 60 min (i = cholesterol, ii = cholesterol 5α, 6α-epoxide or 5β, 6β-epoxide, and iii = 5 7β-hydroxy cholesterol,)

The TLC chromatogram developed from unsaponifiable materials extracted from ghee samples at the beginning of the frying process showed no COPs. However, after 45 minutes of frying, control ghee showed the presence of COP, which was recognized as cholesterol 5α, 6α-epoxide and after 60 minutes of frying, presence of both cholesterol 5α, 6α-epoxide and 7β-hydroxy cholesterol were observed. In BHA and CLE added ghee, COPs (cholesterol 5α, 6α-epoxide) was noticed only after 45 min of frying. These findings suggest that CLE and BHA inhibited the production of COPs in ghee during deep frying for up to 45 minutes. Rahila *et al.* (2018) evaluated that the TLC chromatogram of USM obtained from ghee samples at the beginning of frying did not show any COPs, but control ghee fried for 45 min showed the presence of COPs, which was identified as cholesterol 5a, 6a-epoxide and at 60 min of the frying presence of both cholesterol 5α, 6α-epoxide and 7b-hydroxy cholesterol was shown. In BHA added ghee, COP (cholesterol 5α, 6α-epoxide) was also noticed after 60 min of frying, whereas ghee added with rosemary extract inhibited the oxidation of cholesterol throughout the frying process of 60 min. Cholesterol level in ghee ranges from 0.3 to 0.4 per cent. According to Nath *et al*. (1996) COPs are not present in ghee prepared and kept under normal conditions. Low moisture and high phospholipid content are responsible for ghee's storage stability (Achaya, 1997). The use of ghee for frying for a short time did not result in the creation of cholesterol oxides, but a 15-minute cooking process did. Furthermore, adding ghee residue as a natural antioxidant source delayed the development of COPs for up to 60 minutes of frying (Nath *et al*., 1996).

Conclusion

Curry leaves extract (CLE) was found to be a more effective natural antioxidant than BHA in delaying oxidative degradation in ghee during deep frying. The addition of CLE did not affect the sensory parameters of ghee, and CLE added ghee showed better thermal stability than ghee with BHA and control ghee. The study concluded that CLE could be used as a natural additive to improve the shelf life of ghee during frying, making it a viable alternative to synthetic antioxidants that can have negative effects on human health.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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