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Curry leaf (*Murraya koeneigii*) extract as a natural source of antioxidants for enhancing the oxidative and thermal stability of ghee[#]

Mohammed Arshath[1*], M. P. Rahila[2], M. P. Divya[2], J. L. Smitha[2], S. Athira[3] and P. Sudheerbabu[4]
Department of Dairy Chemistry,
Verghese Kurien Institute of Dairy and Food Technology, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

Ghee is used for making various sweets and dishes in addition to its various culinary purposes. In the course of such preparations, ghee is subjected to different degrees of heat treatment which on combining with air can undergo considerable physico-chemical changes. The high temperature cooking process creates a series of complex chemical reactions in ghee which affects the sensory and the functional properties of foods as well as the ghee used. This leads to adverse health effects on consumers. One of the best ways to protect ghee from autooxidation and thermal oxidation is by the incorporation of antioxidants during their processing. Addition of synthetic antioxidants is restricted in several countries. Scientific investigations have also found that the use of synthetic antioxidants in foods may harm the liver and lead to the development of cancer. The aim of the present study was to check the effect of curry leaves (Murraya koeneigii) extract on the thermal stability of ghee. Based on the peroxide and thiobarbituric acid *(TBA) value determinations, it was observed that quality of ghee samples added with 1 % CLE during frying was significantly higher than that of control (ghee without antioxidant) and ghee added with 0.02 % BHA (a synthetic antioxidant). At 15 minutes of frying (180°C), the peroxide value was 3.615±0.24, 1.04±0.10 and1.07±0.02 mM O_2/kg fat for the control, BHA added and CLE added ghee respectively and this increased to 8.23±0.23, 6.47±0.21 and 4.0±0.01 mM O_2/kg fat after 60 minutes of frying, respectively. Findings suggested that CLE could be used as a source of natural antioxidants for imparting thermal stability to ghee during frying.*

Keywords: *Ghee, curry leaves, sensory characteristics, TBA value, DPPH, thermal stability, deep frying*

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1. *M-Tech Scholar*
2. *Assistant professor*
3. *Assistant professor, Dept of Food Chemistry*
4. *Registrar, Kerala Veterinary and Animal Sciences University*

**Corresponding author: arshadmhd94@gmail.com, Ph: 9645904001*

Ghee is a form of clarified butterfat, traditionally used in Asian cooking just like butter. Due to its good flavour and pleasant aroma, it plays an important role in the Indian diet. Besides being a source of lipid nutrients, it also contains fat-soluble vitamins and essential fatty acids. Ghee is used for making various sweets and dishes due to its characteristic pleasant and rich flavour. In the course of such preparations, ghee is subjected to different degrees of heat treatment in the presence of air and can undergo considerable physico-chemical changes. Such changes in ghee are due to thermal oxidation, hydrolysis and polymerisation reactions. Hydrolysis results in the formation of free fatty acids whereas the oxidation process produces hydroperoxides and small molecular weight carbonyl compounds. This whole process can lead to the formation of polar compounds and the degradation of antioxidants that further degrades frying oil. Eventually through mass transfer process, these degradation products accumulate into fried food and reduce the nutritional quality of both the frying medium and the food. Thus, the study of the thermal stability of ghee is of research interest and calls for detailed systematic analysis. It was in this context that the present study was undertaken.

One of the best ways to protect lipids from autooxidation and thermal oxidation by incorporating antioxidants during their processing. Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and Propyl gallate are the most commonly used synthetic antioxidants permitted in fat rich products by the Food Safety and Standards Authority of India (FSSAI 2011) but these are not permitted for use in ghee. Scientific investigations have proved that the use of synthetic antioxidants in foods may harm the liver and contribute to the development of cancer (Parmar *et al*., 2013). In experimental animals, the use of synthetic antioxidants has resulted in teratogenic, carcinogenic and mutagenic effects (Pawar *et al*., 2012). Hence, the use of synthetic antioxidants is restricted in several countries. These factors have prompted us to explore the use of edible plant resources as a safer alternative. In addition to this, customer demand for natural food ingredients as antioxidants, also favours substantial research in this area. The usage of natural antioxidants in the food industry has exploded in recent years. Many herbs have the capacity to prevent lipid oxidation during food storage, which is mainly mediated by their inherent antioxidant activity; thus the use of herb and spice extracts in milk and milk products is quickly evolving (Pokorny and Korczak, 2001; Pawar *et al*., 2012)

Herbs have long been used both for food and medicinal applications and also have been an important element of human culture from the beginning of time. Secondary vegetal metabolites known as phenolic compounds or polyphenols are a diverse group of phytochemicals with antioxidant properties that contribute to a favourable physiological effect. The current study was undertake with the aim of exploring the use of natural antioxidants for the improvement of the oxidative and thermal stability of ghee. Curry leaves (*Murraya koenigii*) are widely used in southern and west-coast India as a seasoning for cooking. The plant is highly valued for its leaves which are widely used for flavouring and spicing food. Curry leaves are rich in bioactive compounds like polyphenols, alkaloids and flavonoids which show multiple bioactive functions of antioxidant, anticancer, antimicrobial, antidiabetic and hepato-protective nature. In curry leaves, the carbozole alkaloids that have been recently isolated include mahanimbine and koenigine, which possess higher antioxidant activity (Igara *et al*., 2016). Kapadiya and Aparnathi (2017) found that adding the extract of curry leaves to ghee prevented oxidative degradation and that the peroxide value of different fresh ghee samples after 22 days of storage at 80°±2°C was in the order of control > curry leaves (0.3 percent)> BHA (0.02 percent). The present study thus focused on exploring the potential of the Curry leaves extract (CLE) to enhance the oxidative and thermal stability of ghee.

Materials and methods

The University Dairy Plant of Kerala Veterinary and Animal Sciences University, Mannuthy, provided the cow milk cream (40-42% fat) needed for the manufacture of ghee. “From citspray aroma sciences, Nagpur” provided CLE with a minimum of 4.2 per cent

antioxidative substances, expressed as the sum of carnosic acid and carnosol. Chemical analysis was performed using analytical grade chemicals acquired from a variety of reputed suppliers.

Preparation of ghee

Cream with 40-42% fat was collected, cooled to 8–10°C and aged overnight at same temperature. At 10° C, the aged cream was churned into butter, which was then clarified at 110 ± 1° C before being filtered through cheese cloth (Rahila *et al.,*2017*)*. The ghee was then cooled to 60°C before adding the CLE. The amount of CLE added was determined by optimisation studies as discussed below. The control ghee was without CLE. In this investigation, ghee containing 0.02 percent BHA was also used for comparison.

Optimisation of CLE level for addition to ghee

Curry leaf xtract was added to ghee at concentrations of 0.1, 0.25, 0.5, and 1.0 percent, and the samples were judged for sensory quality on a 9-point hedonic scale (range from 9 = like extremely to 1 = dislike greatly) by a trained panel of seven analysts in the sensory evaluation laboratory of the Verghese Kurien Institute of Dairy and Food Technology, Kerala, India.

Analysis of physicochemical parameters of ghee

Ghee samples were analysed for physico-chemical parameters like Moisture (FSSAI, 2015), Colour (IS. 3508, 1966), Acid value (FSSAI, 2015), Saponification value (FSSAI, 2015), Iodine value (FSSAI, 2015), Peroxide value (IS: SP: 18 [Part XI], 1981).

Viscosity

Brookfield viscometer with jacketed small sample adaptor and S18 spindle was used to measure viscosity of ghee samples (Sahasrabudhe *et al*., 2017). Five milliliters of the sample was added to a sample cup and kept for 60 s before measurement was taken and the temperature of the sample was maintained at 31°C. Readings were taken at 50 rpm and the result was expressed in centipoise (cP).

Antioxidant activity of ghee added with CLE

The capacity of antioxidants to quench DPPH (2,2-diphenyl-1-picrylhydrazyl) radical in ghee was determined as per the procedure described by Espin *et al*. (2000) with slight modifications. Ghee sample (0.2 mL) was added to 3.8 mL of ethyl acetate to obtain 4 mL of the mixture, followed by addition of 1 mL of DPPH solution (6.09 $\times 10^{-5}$mol/L solution in ethyl acetate). After 10 min incubation at dark the optical density (OD) was measured at a wavelength of 520 nm using a spectrophotometer (Perkin Elmer Lambda 25 UV/ Visible spectrophotometer). The reference sample used contained 1 mL of DPPH solution in 4 mL ethyl acetate. The per cent radical scavenging activity was calculated as follows:

Percentage radical scavenging activity (% RSA) =

$$\frac{Control\ OD - Sample\ OD}{Control\ OD} \times 100$$

Thiobarbituric acid (TBA) value

Table 1. Sensory analysis of ghee added with different levels of CLE

Sensory scores of ghee added with CLE					
Sensory property	**Level (%) of CLE**				
	0	**0.1**	**0.25**	**0.5**	**1**
Colour and appearance	8.42±.20[a]	8.57±0.20[a]	8.28±0.28[a]	8.28±0.28[a]	8.28±0.18[a]
Flavour	8.35±0.17[a]	8.07±0.202[a]	8.28±0.08[a]	8.21±0.17[a]	8.14±0.14[a]
Body and texture	7.85±0.20[a]	7.85±0.26[a]	7.42±0.20[a]	7.42±0.20[a]	7.57±0.36[a]
Overall acceptability	8.28±0.26[a]	8.28±0.28[a]	7.78±0.23[a]	7.71±0.28[a]	7.71±028[a]

Mean ± SE, n=3

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

The thiobarbituric acid (TBA) value was measured as per the method of Patton and Kurtz, 1951. About 0.1 g of molten ghee samples was accurately weighed into a centrifuge tube, and to this 1 mL of trichloro acetic acid (35%) and 2 mL of TBA reagent (0.36 g of TBA (2-Thiobarbituric acid) and 0.1 g of anhydrous sodium sulphate in 100ml distilled water) were added. The contents were shaken vigorously. The tubes were incubated in a boiling water bath for 15 min. The contents were then cooled, mixed with 1 mL of glacial acetic acid and 2 mL of chloroform and centrifuged at 500 g for 5 min to obtain two clearly separated layers. The optical density (OD) of the supernatant was measured at 532 nm using a spectrophotometer. The results were expressed as TBA reactive substances (TBARS) per 0.1 g of ghee. Blank was also prepared simultaneously without ghee.

Thermal stability of ghee during deep frying

Gulabjamun dough was prepared using a commercial *gulabjamun* ready mix; 100 balls of 6-7 g weight were fried in 1500 g of ghee at 180^{0}C in a stainless-steel deep fryer (AGARO- 33390 Marvel 1700-Watt Deep Fryer). Deep frying was done for 60 min and samples were drown at every 15 min interval and acid value and peroxide value of ghee samples were analysed at room temperature.

Acid value

The acid value of ghee was determined by directly titrating the ghee in an alcoholic medium against a standard potassium hydroxide solution (FSSAI, 2015).

Peroxide value

The usual method of assessment of oxidative rancidity in ghee is by determination of peroxide value (PV) which is reported in units of milliequivalents of peroxide oxygen per kg of fat or ml of 0.002 N sodium thiosulphate per g of sample. The most common method for PV determination is based on iodometric titration described in IS: SP: 18 [Part XI], (1981).

Statistical analysis

Data obtained were expressed as mean values with standard errors. In each analysis, the data obtained from three replications were analysed statistically using analysis of variance (ANOVA) with a significance level of $p < 0.05$, and the comparisons between treatment means were done by Tukey's multiple comparison test in SPSS software (IBM Corporation, Armonk, NY).

Results and discussion

The purpose of this study was to evaluate the effect of CLE on the oxidative and thermal stability of ghee. Ghee without CLE and ghee with BHA (0.02%) were also evaluated for comparison.

Table 2. Physicochemical parameters of ghee

Parameter		Ghee added with		
		Control	BHA (0.02%)	CLE (1.0%)
Moisture (%)		0.17±0.005^a	0.172±0.025^a	0.173±0.004^a
Colour	L*	56.28±0.05^a	56.31±0.05^a	60.12±0.07^b
	a*	1.45±0.01^a	1.48±0.12^a	0.34±0.06^b
	b*	19.85±0.17^a	19.9±0.24^a	36.42±0.42^b
Viscosity (Cp at 31˚C)		36±0.0^a	36.1±0.16^a	35.8±0.16^a
Acid value		0.44±0.0^a	0.44±0.0^a	0.44±0.0^a
Saponification value		232.18	233.29	232.88
Iodine value		32.13	32.37	32.43
Peroxide value (mM O_2/kg fat)		0. ±0.0	0. ±0.0	0. ±0.0
Thiobarbituric acid value (TBARS)		0.028 ±0.0^a	0.025 ±00^a	0.026 ±00^a
Antioxidant activity (%RSA)		30.13±0.08^a	88.13±0.31^b	60.06±0.62^c

Mean ± SE, n=3

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

Table 3. Acid value of ghee samples during deep frying

Acid value of ghee			
Frying interval (min)	Ghee added with		
	Control	BHA (0.02%)	CLE (1.0%)
0	0.44 ± 0.0^{Aa}	0.44 ± 0.0^{Aa}	0.44 ± 0.0^{Aa}
15	0.53 ± 0.05^{Ba}	0.486 ± 0.02^{ABa}	0.46 ± 0.00^{ABa}
30	0.73 ± 0.03^{Ca}	0.55 ± 0.03^{Bbc}	0.52 ± 0.00^{Bc}
45	0.85 ± 0.01^{Da}	0.82 ± 0.00^{Cab}	0.72 ± 0.00^{Cc}
60	1.11 ± 0.06^{Ea}	0.97 ± 0.00^{Db}	0.87 ± 0.00^{Dc}

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

Table 4. Peroxide value of ghee samples during deep frying

Peroxide value of ghee (mM O_2/kg ghee)			
Frying interval (min)	Ghee added with		
	Control	BHA (0.02%)	CLE (1.0%)
0	0 ± 0.00^{Aa}	0 ± 0.00^{Aa}	0 ± 0.00^{Aa}
15	3.615 ± 0.24^{Ba}	1.04 ± 0.10^{Bb}	1.07 ± 0.02^{Bb}
30	5.29 ± 0.33^{Ca}	2.165 ± 0.15^{Cb}	1.90 ± 0.02^{Cb}
45	6.8 ± 7.12^{Da}	3.78 ± 0.08^{Db}	2.57 ± 0.02^{Dc}
60	8.23 ± 0.23^{Ea}	6.47 ± 0.21^{Eb}	4.0 ± 0.01^{Dc}

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

Optimisation of CLE level for addition to ghee

Ghee samples were added with different levels of CLE (0, 0.1, 0.25, 0.5 and 1.0 percent) and sensory qualities were studied in a preliminary trial to optimise the level of CLE that could be added to ghee. Table 1 summarises the findings. In comparison to ghee without CLE, adding 1.0 percent CLE had no significant effect on the sensory quality of ghee in terms of colour and appearance, flavour, body and texture and overall acceptability. So ghee with 1 % CLE was selected for further analysis.

Physicochemical parameters of ghee samples

The ghee (Control, BHA (0.02%) and CLE (1.0%)) samples were analysed for their physicochemical parameters. The data obtained revealed that there is no significant change ($P<0.05$) in moisture, viscosity, acid value, saponification value, iodine value, peroxide value and TBA value of ghee samples (Table 2 and these values are also within the standards prescribed by regulatory authorities.

HunterLab MSEZ colorimeter was used for the estimation of colour of ghee. Addition of 1 % CLE significantly changed the instrumental colour features of ghee as compared to control and BHA added ghee. This change in L*, a* and b* values of CLE added ghee may be due to the presence of chlorophyll molecules in the CLE.

The ability of CLE to quench free DPPH radicals was used to determine its antioxidant potential. The amount of DPPH absorbance reduction in the presence of antioxidants is related to the antioxidant's ability to scavenge free radicals. The radical-scavenging activity of control ghee, ghee with BHA (0.02%) and ghee with 1.0 percent CLE was measured at 200 ppm in the DPPH system, and the findings are

shown in Table 2. It clearly states that addition of CLE at 1 % level significantly improved the antioxidant activity of ghee and is much better than that of 0.02% BHA.

Bipinbhai and Aparnathi (2017) reported that methanolic extract of curry leaves showed an antioxidant activity of 84.07±0.93% in terms of DPPH radical scavenging activity. In a study by Lalwani *et al*. (2014) curry leaves extract (ethanolic) demonstrated an 81.13 % free radical scavenging activity at a concentration of 0.02 mg DPPH per ml ethanol. Major components found in curry leaves include cis-ocimene (34.1%), β- caryophyllene (35.8%), α-pinene (19.1%), δ- terpenene (6.7%) and β-phellandrene (2.55%) and linalool (8.0%) (Ganesan *et al*., 2013). Aryl hydroxyl group of this alkaloids in curry leaves constitutes higher antioxidant potential.

Thermal stability of ghee during deep frying

Ghee is used as a medium for deep fat frying and samples were drawn directly from the fryer in each 15 min interval for analysing its thermal oxidation.

Acid value

The acid value of an oil is a measurement of the amount of free fatty acids (FFA) present in it. Fatty acids are normally found in the triglyceride form; however, they can be degraded into free fatty acids during processing. The greater the acid value, the higher the FFA level, which correlates to lower oil quality. Table 3 depicts the variations in the acid values of control ghee, ghee containing CLE and ghee with BHA during deep frying. During deep frying, the acid value in the ghee samples was found to increase considerably ($p<0.05$). There was no significant difference ($p<0.05$) observed on the acid value of control and ghee added with antioxidants at the beginning of frying as the added antioxidants did not contribute to any ghee acidity. After 30 minutes of frying, all samples had a significant increase in acid value (0.730±.03, 0.550±.03, and 0.520±.00 for control, BHA added and CLE added ghee, respectively). The acid value of ghee samples again increased to 1.11±0.06, 0.97±0.00, and 0.87±0.00 for control, BHA added and CLE added ghee respectively, towards the end of frying. This increase in acid value of ghee used for deep frying was least for ghee added with 1 % CLE as compared to BHA added and control ghee. In a similar study using a mixture of sunflower oil and rice bran oil, demonstrated good thermal stability during deep fat frying cycles. This had a substantially lower acid value than pure sunflower oil after six frying cycles and it may be due to the presence of antioxidants (gamma-oryzanol) from rice bran oil (Sharma *et al*., 2006).

Peroxide value

The effects of CLE and BHA on the formation of peroxides during frying were evaluated, and the results are shown in Table 4. After first 15 minutes of frying, the peroxide value of ghee samples was 3.615±0.24, 1.04±0.10 and 1.07±0.02 mM O_2/kg fat for control, BHA and CLE, respectively. Peroxide values increased to 8.23±0.23, 6.47±0.21 and 4.0±0.01 mM O_2/kg fat after 60 minutes of frying, respectively. This indicates that addition of 1 % CLE improved the thermal stability of ghee at 180°C. Further the antioxidative capacity of CLE was higher than that of 0.02% BHA at elevated temperature.

The breakdown of antioxidant components in BHA during the frying process may account for their lower antioxidant properties. Many researchers have noted that BHA's antioxidant activity may be reduced at high temperatures (Lee and Kim, 1979; Warner *et al*., 1986). Many studies have found that BHA volatilises during frying and is ineffective in improving frying oil oxidative stability (Warner and Gehring, 2009).

Conclusion

The goal of this study was to evaluate the potential of using curry leaves (*Murraya koenigii*) an easily available herb as a natural additive to improve oxidative and thermal stability of ghee during deep frying. Adding CLE to ghee imparts improved oxidative stability than control ghee. Curry leaves extract

(1.0 percent) was found to be more effective than BHA (0.02 percent) for delaying oxidative degradation in ghee during deep frying. Hence, it can be concluded that curry leaves can be utilised as a natural antioxidant to reduce the thermal degradation of ghee while frying and would be favoured over BHA in order to reduce 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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