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Evaluation of curry leaves (*Murraya koenigii*) for enhancing shelf-life of ghee against oxidative deterioration

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Abstract
Curry leaves plant is native to India and commonly consumed in various types of Indian dishes so curry leaves was used as an additive in ghee and its antioxidant potential was evaluated to enhance the shelf-life of ghee. Curry leaves was analysed for its total phenolic content and radical scavenging activity. Curry leaves contained 1913.33±57.35 mg GAE/100g total phenolic content and gave 84.07±0.93% DPPH radical scavenging activity. Addition of curry leaves at final stage of heat clarification were found more effective than at initial stage of heat clarification. Optimum rate for use of curry leaves in treatment of ghee was found 0.3 per cent. Curry leaves was found to be capable of retarding oxidative degradation in ghee but were less effective than BHA.

Keywords: curry leaves (*Murraya koenigii*), antioxidant, peroxide value, flavour score

Introduction
Murraya koenigii commonly known as curry plant belongs to the family Rutaceae. The plant is a medicinal plant and native to India, Sri Lanka and other South Asian countries. Curry leaves or sweet neem leaves are used in a variety of Indian dishes. The plant is highly valued for its leaves which are used for flavouring and spicing of food. Leaves are rich in many bioactive compounds like polyphenols, alkaloids and flavonoids which showed multiple bioactive functions like antioxidant, anticancer, antimicrobial, antidiabetic and hepatoprotective. Higher concentration of the bioactive compounds seen in plants leaves showed higher antioxidant activities. However, the antioxidant potential in turn depends on the total polyphenolic compounds, essential oils and other compounds. In curry leaves, the carbozole alkaloids that are recently isolated are of mahanimbine and koenigine, which showed higher antioxidant activities (Igara *et al.,* 2016) [1]. The aryl hydroxyl group of alkaloids in curry leaves showed higher antioxidant potential. Recently Mitra *et al.* (2012) [2] reported that curry leaves showed higher antioxidant activity in cadmium induced oxidative stress in rats. The chemical composition of the essential oil from leaves of *M. Koenigii* varies with variation in agro climatic and geographical variation. Monoterpenoids and its oxygenated derivatives are the main constituents of essential oil. Leaves constitute major components such as 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) [3].

Ghee under ambient conditions of storage undergoes oxidative deterioration. Addition of synthetic antioxidants is common approach to extend shelf-life of ghee, but use of synthetic antioxidants which posse potential health risk necessitated attention towards natural antioxidants. Herbs are considered very good sources of natural antioxidants, but very limited work has been reported for utilization of herbs as a possible antioxidant in ghee. Some limited work is reported for utilization of their extracts prepared in organic solvents in ghee (Patel *et al.,* 2013) [4]. Minor components are critical to the activity due to their synergistic effect might be lost during preparation of extracts using organic solvent. Even addition of small amount of extracts gave high pungency to the product, but no work is reported for utilization of herb as such in ghee. This research aims to evaluate the potential of curry leaves (*Murraya koenigii*) as an antioxidant to enhance shelf-life of ghee against oxidative deterioration. Keeping this idea as a central goal, the study was divided into four phases (a) Assess the compatibility of curry leaves as an additive in ghee, (b) select the stage of addition of curry leaves in preparation of ghee, (c) optimize the rate of curry leaves in preparation of ghee, (d) comparison of curry leaves with synthetic antioxidant (BHA).

Materials and methods

Chemicals and glassware

All the chemicals and glassware used in the present study were of analytical (AR) grade and standard quality supplied by authorized dealers.

Collection and preparation of plant material

Curry leaves (*Murraya koenigii*) was brought from Directorate of Medicinal and Aromatic Plants Research, BACA, AAU, Anand, Gujarat, India. Leaves were cleaned to remove dirt and damage parts then they were vaccum dried in anubhav dairy, AAU, Anand. After drying, dried leaves were converted into coarse size particles and transferred in to zip lock plastic cover, then put in air tight plastic bottle and stored at refrigeration temperature. Fresh leaves and dried leaves of curry leaves are shown in Figure 1.

Fresh leaves Dried leaves

Fig 1: Fresh leaves and dried leaves of curry leaves

Evaluation of curry leaves for its antioxidant potential

1. Preparation of extract

0.5 g powder of curry leaves was treated with 10 ml of methanol-water (8:2, v/v) in a shaking water bath at 35°C for 24 h as described by Song *et al*. (2010) [5]. The mixture was then centrifuged at 4,000 rpm for 10 min. The supernatant (curry leaves extract) was recovered for the determination of the total phenolic content and radical scavenging activity.

2. Analysis of total phenolic content

Total phenolic content of curry leaves extract was analysed by Folin-Ciocalteu (FC) reagent according to the procedure described by Singleton and Rossi (1965) [6]. 0.05 ml sample of curry leaves extract was taken in a test tube and volume was made up to 1 ml with distilled water. To this 0.5 ml each of diluted FC reagent (1:1) and 10 ml 7.5 % sodium carbonate solution were added. The contents were mixed using vortex mixer. This was incubated under dark at room temperature for 30 min. For blank preparation 1 ml of distilled water was taken instead of sample. The absorbance was measured against blank at 750 nm using spectrophotometer. The result was expressed in terms of mg of GAE per 100 gm of dried curry leaves.

3. Radical-Scavenging activity by DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) assay

The radical-scavenging activity of curry leaves extract was determined as the ability to scavenge DPPH radicals according to the procedure of Brand-Williams *et al*. (1995) [7]. 0.05 ml of sample of curry leaves extract was taken in a test tube and the volume was made up to 1 ml with methanol. 3 ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 37ºC for 15 min. in a water bath. The control was prepared by taking 1 ml methanol and 3 ml of 0.1 mM methanolic DPPH solution. Methanol was used (as blank) for the baseline correction. Absorbance of the control and sample were measured at 517 nm using a spectrophotometer against blank (methanol). DPPH activity was expressed as the inhibition percentage and was calculated using the following formula.

Radical-scavenging activity (% inhibition) = [(Ac – A)/Ac] x 100

Where, Ac = Absorbance of control and A = Absorbance of sample

Preparation of ghee and addition of curry leaves

White butter was procured from a commercial dairy plant of Amul Dairy, Anand. Ghee was prepared by creamery butter method described by De (2004) [8]. Ghee was clarified at 120ºC temperature. Prepared ghee was divided into two portions of 100g each. To portion 1 nothing was added which served as control. To the second portion, curry leaves was added at the rate of 0.5% (w/w) respectively. The mixtures of ghee and curry leaves were thoroughly mixed using glass rod and allowed to stand at 80ºC for 30 min. in a hot air oven. The treated ghee samples were filtered through 6 layers of muslin cloth. The ghee samples were assessed for its compatibility in ghee stored. The colour characteristic of ghee samples was also observed.

Selection of stage to add curry leaves in preparation of ghee

For selection of stage for addition of herbs in preparation of ghee, curry leaves was added at two different stages during manufacture of ghee.

(1) Addition at the initial stage of heat clarification of butter (in melted butter)

(2) Addition at the final stage of heat clarification of butter (105ºC temperature)

For evaluating two different stages in preparation of ghee (initial stage of clarification and final stage of clarification) for treatment with the curry leaves, butter sample (120 g) was taken in to each of three of 500 ml glass beakers. The beakers containing butter were arranged in round shaped sand bath at equal distance from the centre of the sand bath. Sand bath was heated by gas fired burner. When butter was melted completely curry leaves was added in 1 beaker. In another beaker curry leaves was added when their temperature reached to 105°C (nearer to heat clarification). One sample was not treated with any curry leaves to serve as a control. The heating was continued till temperature reached to 120°C. In the entire process of ghee preparation each samples were mixed with stainless steel spatula and then content of each beaker was filtered through 6 folded muslin cloth, ghee was collected in 150 ml glass beakers and stored in incubator at 80°±2°C. Sand bath was used to prepare ghee samples simultaneously under similar and uniform heating conditions. Total four replications were conducted.

The ghee samples were analysed for peroxide value when fresh and at an interval of 2 days for 12 days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale. The stage for addition of curry leaves giving better stability against oxidative deterioration of ghee was selected for further study.

Optimization of rate for addition of curry leaves in preparation of ghee

Curry leaves was added according to results obtained from the selected stage at the rate of 0.1, 0.2, 0.3 and 0.4% (expected yield of ghee from butter). The curry leaves treated ghee was prepared as per above method. The sample of ghee without addition of curry leaves was also prepared to serve as control.

All the 5 ghee samples were stored in an incubator at 80º±2ºC. The ghee samples were analysed for peroxide value when fresh and at an interval of 2 days for 12 days then after at an interval of 1 day for 22 days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale. Total three replications were conducted. The rate at which curry leaves giving better stability against oxidative deterioration of ghee was selected for further study.

Comparison of curry leaves with synthetic antioxidant (BHA)

In this phase, ghee samples were prepared with curry leaves at the selected stage and optimized rate on the basis of expected yield of ghee from butter as per procedure described in phase b (selection of stage). Simultaneously, butylated hydroxyl anisole (BHA) was added directly into the freshly prepared ghee at the rate of 0.02% (w/w). The sample of ghee without addition of curry leaves served as a control. All the 3 ghee samples were stored in an incubator at 80º±2ºC. Oxidative changes taking place in ghee were monitored by analysing the ghee samples for peroxide value when fresh and at an interval of 2 days for 22 days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale. Total three replications were conducted.

Determination of oxidative changes in ghee

1. Peroxide value of ghee

The peroxide value of ghee was determined by the method (Iodometric method) as described in IS: SP: 18 (part XI) (1981) [9].

2. Sensory evaluation of ghee

All samples of ghee made in the laboratory were evaluated for their sensory characteristics on a 9-point hedonic scale by a panel of 9 experienced judges. Sensory evaluation was developed considering the rancidity of ghee. The nine experienced judges, who were familiar with rancidity (off-flavour) of ghee, were academic staff aged 30 to 56 years. Each judge evaluated the ghee for flavour score (i.e. rancidity) using the 9-point hedonic scale.

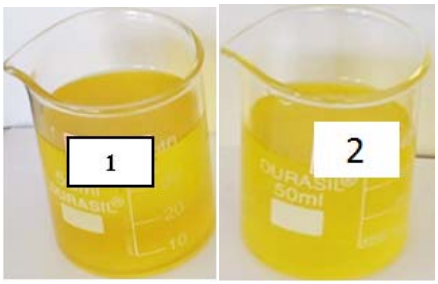
Statistical analysis

The collected data were subjected to statistical analysis. Data were analysed by completely randomized design and critical difference test at 5% level of significance ($p<0.05$) as per the procedure mentioned by Steel and Torrie (1980) [10].

Results and discussion

The first and foremost point for consideration was the compatibility of curry leaves to be used for study. Curry leaves was added at the rate of 0.5% w/w of ghee and then compatibility of curry leaves added ghee sample was assessed based on their flavour. Colour characteristic of curry leaves added ghee sample was also examined by visual observation. The results indicated that all the samples of ghee treated with herbs were found acceptable for their flavour in sensory evaluation by members of the panel of the 9 judges.

It was evident from the examination of the colour characteristic of ghee samples that both the ghee samples (curry leaves added ghee sample + control ghee sample) acquired golden yellow colour. Colour of curry leaves did not impart any objectionable colour to the ghee. The colour characteristic of the fresh ghee samples (control ghee and curry leaves added ghee) is presented in Figure 2.



Control ghee Curry leaves added ghee

Fig 2: The colour characteristic of the fresh ghee samples

Evaluation of curry leaves for its antioxidant potential

Since use of curry leaves was found compatible in ghee, it was evaluated for its antioxidant potential. To evaluate the antioxidant potential, curry leaves was analysed for its total phenolic content and radical scavenging activity. Total three replication were conducted. Curry leaves contained 1913.33±57.35 mg GAE/100 g of curry leaves total phenolic content and gave 84.07±0.93% DPPH radical scavenging activity.

Almey *et al.* (2010) [11] determined the level of total phenolic compounds in curry leaves and found that both methanolic and ethanolic extracts of *Murayya koeniigi* had 20.46±0.20 and 12.31±0.18 mg GAE per g phenolic content respectively. Ishtiaque *et al.* (2015) [12] determined the total phenolic content and antioxidant activity of methanolic extract of *Murraya koenigii*. Result from their study indicated that curry leaves contained 170 mg GAE per 100g total phenolic content and 20-64% DPPH activity. In a work carried out by Lalwani *et al.* (2014) [13], curry leaves extract (ethanolic) at a concentration of 0.02mg DPPH per ml ethanol exhibited the free radical scavenging activity of 81.13%.

The total phenolics content and radical scavenging activity of curry leaves evaluated in the study was within the range reported in the literature. However, some deviations in total phenolics content might be attributed to variations variety of the herbs (Benabdallah *et al.*, 2016; Abdulkadir *et al.*, 2015) [14, 15], prevailing agroclimatic conditions of the area in which herb is grown (Jaiswal *et al.,* 2014) [16], agronomic practises followed in the herb farming (Azhar *et al*., 2011; Pakade *et al.,* 2013) [17,18], maturity of the herb at the stage of harvesting (Jinesh *et al.,* 2010) [19], method followed for post-harvest processing of the herb (Pakade *et al.,* 2013; Al-juhaimi and Ghafoor, 2011) [18,20], the type and concentration of solvent (Hasim *et al.*, 2015; Wangcharoen and Gomolmanee, 2011) [21,22] as well as polarity of solvent (Rahman *et al.*, 2013; Basak *et al*., 2014) [23,24] used for analysis, the method followed for the estimation of the total phenolics content (Jinesh *et al.,* 2010; Wangcharoen and Gomolmanee, 2011; Rahman *et al.*, 2013) [19, 22, 23], etc. However, some deviations in radical scavenging activity might be attributed to variations in chemical composition of the different herbs with respect to phenolics and other antioxidants as well as pro-oxidants content (Tupe *et al.,* 2013; Kaur and Mondal, 2014; Fukumoto and Mazza, 2000; Bouayed and Bohn, 2010) [25, 26, 27, 28].

Selection of stage to add curry leaves in preparation of ghee

Ghee is almost anhydrous milk fat and obtained by clarification of milk fat at high temperature usually at 110 to 120°C temperature (Ganguli and Jain, 1973) [29]. Exposure of the herbs to such a high temperature may adversely affect the stability of major and/ or minor antioxidant components

present in the herbs. Even possibility also exist about interaction of antioxidants present in herbs with ghee residue, leading to decrease in effectiveness of the antioxidants. At the same there is possibility of improvement in extraction of these antioxidants due to their better leaching from the herbs to ghee at higher temperature. Therefore, it is necessary to find out appropriate stage for addition of herb in process method adopted for preparation of ghee. From examination of manufacturing process for ghee it can be envisaged that there are three possible stages to add herbs into ghee, as listed below.

(1) Initial stage of heat clarification of butter in to ghee (in melted butter)

(2) Final stage of heat clarification of butter in to ghee (near 105°C temperature)

(3) After the heat clarification and separation from ghee residue (in hot ghee at 80°C)

Adopting the first and second possibility is the most appropriate in the study particularly when small number of samples to be treated alike, to avoid sample to sample variation in intensity of heat clarification. Sand bath was used to prepare ghee samples simultaneously under similar and uniform heating conditions.

1. Performance of curry leaves added at two different stages in ghee preparation

Effect of two different stages of addition on performance of curry leaves for retarding oxidative deterioration of ghee was measured in terms of changes in peroxide value and flavour score of ghee during storage at 80°±2°C.

1.1 Peroxide value of curry leaves treated ghee during storage

The changes in peroxide value of different ghee samples (control and treated with curry leaves) during storage at 80°±2°C are presented in Table 1 and graphically presented in Figure 1.

Table 1: Changes in peroxide value of curry leaves treated ghee during storage

Storage period (days)	Peroxide value of ghee during storage at 80°±2°C (meq of O_2 per kg fat)		
	Control	Curry leaves added at initial stage of clarification	Curry leaves added at final stage of clarification
0	0.19	0.62	0.49
2	0.53	2.30	1.37
4	1.44	4.02	2.66
6	1.93	5.86	4.23
8	4.04	8.08	5.49
10	6.19	11.44	6.47
12	9.33	14.76	8.13
Source of variation	**Storage period (P) (days)**	**Treatment (T) (Stage of addition)**	**Interaction (P×T)**
SE m	0.42	0.27	0.73
CD (0.05)	1.18	0.77	2.05
CV%	30.59		

The peroxide value of different fresh ghee samples was in the order of control ghee < curry leaves treated ghee at final stage of clarification < curry leaves treated ghee at initial stage of clarification. However, the order of peroxide value of different ghee samples was just reversed at the end of the 12 days storage at 80°±2°C. The order of peroxide value of different ghee samples was just reversed at the end of the storage was curry leaves treated ghee at final stage of clarification < control ghee < curry leaves treated ghee at initial stage of clarification.

It was revealed from statistical analysis that different stage in preparation of ghee used for treatment of ghee with the curry leaves differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. Similarly, period of storage also differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. The interaction effect indicated that the stage in preparation of ghee used for treatment with curry leaves and period of the storage differed significantly from each other in their effect on peroxide value of ghee over a period of storage. Thus, it became evident that the effect of stage in preparation of ghee used for treatment with curry leaves and period of storage were dependent on each other.

The peroxide value of all the three types of ghee samples increased at a steady rate up sixth day of the storage. The rate of rise in peroxide value became steep from 6th day onwards in case of control ghee and that in ghee treated with curry leaves at initial stage of clarification. On the other hand in ghee treated with curry leaves at final stage of clarification, no steep rate of rise in peroxide value was noticed at any stage during the entire storage period.

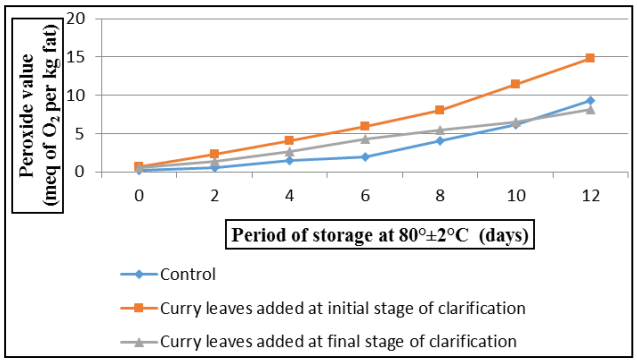


Fig 1: Changes in peroxide value of curry leaves treated ghee during storage

Among the fresh ghee samples (0 day) peroxide value of control ghee was statistically at par with curry leaves treated ghee samples, irrespective of their stage of addition in preparation of ghee. Moreover, peroxide value of control ghee remained significantly ($P<0.05$) lower than that of the curry leaves treated ghee samples at initial stage of clarification during entire period of storage. Similarly, up to 10th day of storage peroxide value of control ghee also remained lower than that of the curry leaves treated ghee samples at final stage of clarification, but their difference was statistically at

par on 10th day of storage. However, peroxide value of control ghee became significantly (P<0.05) higher than that of curry leaves treated ghee samples at final stage of clarification on 12th day of the storage. Peroxide values of both ghee samples were statistically at par only on 0 day. Thereafter peroxide values of ghee sample treated with curry leaves at initial stage of clarification were significantly (P<0.05) higher than ghee sample treated with curry leaves at final stage of clarification throughout the storage period.

From the forgoing resume it became evident that treating the ghee with curry leaves at final stage of clarification reduced the peroxide formation more effectively compared to the ghee with curry leaves at initial stage of clarification. The examination of the results clearly suggested that the treatment of ghee with curry leaves at initial stage of clarification adversely affected the stability of the ghee against oxidative deterioration, due to strong pro-oxidant effect, when compared with the results of the control ghee sample. The careful comparison of the data also suggested that even treatment of ghee with curry leaves at final stage of clarification also had adverse effect the stability of the ghee against oxidative deterioration of the ghee up to 8th day of storage, when compared with the results of the control ghee sample. On 12th of storage ghee with curry leaves at final stage of clarification showed slightly lower peroxide value compared to that of the control ghee sample.

No report is available in the literature for evaluating effect of treatment of ghee with curry leaves at different stages in preparation of ghee. Therefore, results obtained in this study could not be compared as such with the reports in the literature. Though Patel and Rajorhia (1979) [30] carried out the study dealing with the antioxidative effects of curry leaves (*Murraya koeniji*). These authors added the curry leaves to only melted butter (*i.e.* during initial stage of clarification), but they have not carried the study about final stage of clarification.

1.2 Flavour score of curry leaves treated ghee during storage at 80°±2°C

The changes in flavour score of different ghee samples (control and treated with curry leaves) during storage at 80°±2°C are presented in Table 2 and graphically presented in Figure 2.

Table 2: Changes in flavour score of curry leaves treated ghee during storage

Storage period (days)	Flavour score of ghee during storage at 80°±2°C (Out of 9)		
	Control	Curry leaves added at initial stage of clarification	Curry leaves added at final stage of clarification
0	9.00	8.03	8.80
2	8.58	7.45	8.30
4	7.75	6.85	7.88
6	6.60	6.10	7.35
8	5.60	5.38	6.93
10	4.10	4.15	6.57
12	2.40	2.10	6.18
Source of variation	**Storage period (P) (days)**	**Treatment (T) (Stage of addition)**	**Interaction (P×T)**
SE m	0.06	0.04	0.10
CD (0.05)	0.17	0.11	0.29
CV%	3.12		

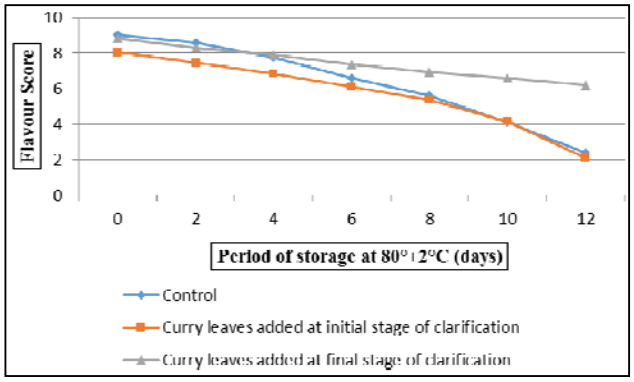


Fig 2: Changes in flavour score of curry leaves treated ghee during storage

The flavour score of different fresh ghee was in the order of control ghee > ghee treated with curry leaves at final stage of clarification > ghee treated with curry leaves at initial stage of clarification. However, the order of flavour score of different ghee samples was just reversed at the end of the 12 days at 80°±2°C. The order of flavour score of different ghee samples was just reversed at the end of the storage was ghee treated with curry leaves at final stage of clarification > control ghee > ghee treated with curry leaves at initial stage of clarification.

The flavour score of all the three types of ghee samples decreased at a steady rate up 4th day of the storage. The rate of decline in flavour score of control ghee sample became steep from 6th day onwards. However, in case of ghee treated with curry leaves at initial stage of clarification as well as at final stage of clarification no steep rate of decline in flavour score was noticed at any stage during the entire storage period. The flavour score of control ghee sample went below the acceptable level (<6) on 8th day of the storage. Similarly, the flavour score of ghee sample treated with curry leaves at initial stage of clarification also went below the acceptable level on 8th day of the storage. However, flavour score of ghee samples treated with curry leaves at final stage of clarification remained acceptable even on 12th day of the storage.

It was revealed from statistical analysis that different stage in ghee preparation used for treatment of ghee with the curry leaves differed significantly (P<0.05) in their effect on changes in flavour score of ghee. Similarly, period of storage also differed significantly (P<0.05) in their effect on changes in flavour score of ghee. The interaction effect indicated that the stage in preparation of ghee used for treatment with curry leaves and period of the storage differed significantly from each other in their effect on flavour score of ghee over a period of storage. Thus, it became evident that the effect of stage in preparation of ghee used for treatment with curry leaves and period of storage were dependent on each other.

Among the fresh ghee samples (0 day) control sample of ghee had significantly (P<0.05) higher flavour score compared to samples of ghee treated with curry leaves, irrespective of their stage of addition in preparation of ghee. Even on 2nd day of storage flavour score of control sample of ghee had significantly (P<0.05) higher flavour score compared to samples of ghee treated with curry leaves, irrespective of their stage of addition in preparation of ghee. Moreover, control ghee had statistically higher (P<0.05) flavour score compared to sample of ghee treated with curry leaves at initial stage of clarification during the entire storage period. However only on 10th day of storage flavour score of control ghee sample was statistically at par with sample of ghee treated with curry leaves at initial stage of clarification. However, from 6th day of the storage flavour score of control ghee sample became significantly (P<0.05) lower than that of sample of ghee treated with curry leaves at final stage of clarification.

Among the sample of ghee treated with curry leaves at two different stages of clarification (initial and final) the flavour score of ghee sample treated with curry leaves at final stage of clarification remained higher throughout the storage period than that ghee sample treated with curry leaves at initial stage of clarification. From the forgoing resume it became evident that treating the ghee with curry leaves at final stage of clarification reduced the peroxide formation more effectively compared to the ghee with curry leaves at initial stage of clarification.

No report is available in the literature for evaluating effect of treatment of ghee with curry leaves at different stages in preparation of ghee on changes in flavour score of ghee during storage. Therefore, results obtained in present study could not be compared as such with the reports in the literature. Though Patel and Rajorhia (1979) [30] carried out the study dealing with the antioxidative effects of curry leaves (*Murraya koeniji*). These authors added the curry leaves to only melted butter (*i.e.* during initial stage of clarification), but they have not carried the study about final stage of clarification.

Kim *et al.* (2006) [31] studied the effect of heat on the antioxidant capacity of grape seed extracts and came to the conclusion that the antioxidant capacity of these extracts increased through the liberation of phenolic compounds by heat. Hence the additive effects are shown well by partitioning easily and spreading evenly well in favourable medium provided by heating. Jeong *et al*. (2004) [32] evaluated effect of heat treatment on the antioxidant activity of extracts from *Citrus unshiu* peels (CP). Their results indicated that the antioxidant activity of CP extracts was significantly affected by heating temperature and duration of treatment on CP. This means that phenolic compounds with antioxidant activity in plants present several kinds of bound states, and a simple heating process can be used as a tool for increasing the antioxidant activity of CP. The TPC in extracts of CP significantly increased by heat treatments.

Better performance of curry leaves used in treatment of ghee at final stage of clarification during preparation of ghee might be attributed to increased liberation of phenolic compounds, easy partitioning, spreading evenly and formation of polyphenols with an intermediate oxidation state of antioxidants by heat. Thus, findings of present study were in corroboration with findings reported and views expressed by various authors as presented above.

Optimization of rate for addition of curry leaves in preparation of ghee

The typical aroma and taste associated herbs in turn adversely affect the organoleptic property of the products like ghee, because people are not accustomed with it. Moreover, it has been reported that the effect of antioxidant depends on its concentration, both at low and high concentration they may become pro-oxidant (Gordon, 1990) [33]. Therefore, it was essential to optimize rate of addition of the selected curry leaves for use in treatment of ghee.

1. Effect of rate of curry leaves used in treatment of ghee

Effect of different rates of addition of curry leaves on its performance in retarding oxidative deterioration of ghee was measured in terms of changes in peroxide value and flavour score of ghee during storage at 80°±2°C.

1.1 Changes in peroxide value of curry leaves treated ghee during storage at 80°±2°C

The results obtained for rate of curry leaves addition on changes in peroxide value of ghee during storage are presented in Table 3 and the trend is presented in Figure 3.

Table 3: Changes in peroxide value of ghee curry leaves during storage after treating with at different rates

Storage period (days)	Peroxide value of ghee during storage at 80°±2°C (meq of O_2 per kg fat)				
	Rate of curry leaves used in treatment of ghee				
	0.0%	0.1%	0.2%	0.3%	0.4%
0	0.19	0.57	0.31	0.19	0.39
2	0.77	0.90	0.85	0.88	0.97
4	1.67	1.46	1.23	1.15	1.42
6	3.68	2.56	1.96	1.88	1.87
8	4.73	3.51	2.11	2.17	2.48
10	6.71	3.72	2.54	2.50	3.32
12	8.71	4.14	3.08	3.54	4.02
13	10.88	4.46	3.68	3.77	5.66
14	12.79	7.06	4.53	4.78	7.82
15	14.72	8.50	6.53	6.33	9.64
16	17.41	8.67	7.85	6.63	9.59
17	20.35	13.04	8.00	7.03	9.53
18	24.71	15.36	8.30	7.73	11.14
19	27.76	15.64	8.70	8.07	11.43
20	31.02	18.10	11.70	8.43	12.12
21	32.97	21.70	15.70	8.91	13.87
22	35.80	27.56	17.67	9.44	15.83

Source of variation	Storage period (P) (days)	Treatment (T) (Rate of addition)	Interaction (P×T)
SEm	0.37	0.20	0.82
CD (0.05)	1.03	0.56	2.30
CV%	16.79		

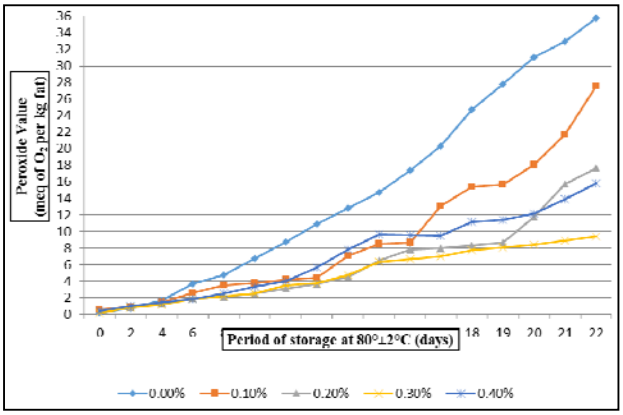


Fig 3: Changes in peroxide value of ghee during storage after treating with curry leaves at different rates

The peroxide value of different fresh ghee sample was in the order of control ghee = ghee treated with 0.3% curry leaves < ghee treated with 0.2% curry leaves < ghee treated with 0.4% curry leaves < ghee treated with 0.1% curry leaves. However, position of control ghee sample changed in the order of peroxide value of different ghee samples at the end of the 22 days storage at 80°±2°C. At the end of the storage the order of peroxide value of different ghee samples was ghee treated with 0.3% curry leaves < ghee treated with 0.4% curry leaves < ghee treated with 0.2% curry leaves < ghee treated with 0.1% curry leaves < control ghee.

The control ghee sample showed steeped rise in peroxide value on 16th day of the storage. Whereas, samples of ghee treated with curry leaves at 0.1, 0.2, and 0.4 per cent rate showed steeped rise in peroxide value on 17th, 18th and 20th day of the storage. Interestingly, sample of ghee treated with 0.3 per cent curry leaves peroxide value of ghee increased at a steady rate, no sharp rise in peroxide value was observed in entire period of storage.

Among the fresh ghee samples (0 day) control ghee sample had lower peroxide value compared to samples of ghee treated with curry leaves at different rate of addition except 0.3 per cent treated ghee sample. However, while remaining entire period of storage peroxide value of control ghee sample also remained higher compared peroxide value of all the curry leaves treated ghee samples. The peroxide value of control ghee became significantly ($P<0.05$) higher compared peroxide value of all the curry leaves treated ghee samples (at the rate of 0.1, 0.2, 0.3 and 0.4 per cent) from 6th day of storage and on subsequent storage up to end.

It was revealed from statistical analysis that different rate of curry leaves used in treatment of ghee differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee during storage. Similarly, storage period also differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. The interaction effect indicated that the rate of curry leaves used in treatment of ghee and period of storage differed significantly from each other in their effect on peroxide value of ghee over a storage period. Thus, it became evident that the effect of rate of curry leaves used in treatment of ghee and storage period were dependent on each other.

Among the sample of ghee treated with curry leaves at different rate of addition (0.1, 0.2, 0.3 and 0.4 per cent), the peroxide value of ghee treated with 0.1, 0.2 and 0.4 per cent curry leaves remained higher than that of the treated with 0.3 per cent curry leaves when fresh and also during almost entire period of their storage. Though, peroxide values of curry leaves treated ghee samples was statistically at par up to 4th day of the storage, then after in remaining storage period peroxide values of ghee sample treated with 0.3 per cent curry leaves was significantly ($P<0.05$) lower than that of sample of ghee treated with 0.1 and 0.4 per cent curry leaves. Sample of ghee treated with 0.2 per cent curry leaves was statistically at par with 0.3 per cent curry leaves added ghee sample up to 15th day of the storage. It can be sum-up that treatment of ghee with curry leaves at four different rates used in the treatment of ghee, all the 4 rates were able to reduce the peroxide formation in ghee during storage 80°±2°C, compared to formation of peroxide in control sample of ghee. However, best control in formation of peroxide in ghee during storage was given by 0.3 per cent curry leaves. Any increase or decrease in the rate of curry leaves from 0.3 per cent resulted in to reduction in effectiveness of the treatment in controlling the peroxide formation in ghee during storage 80°±2°C.

From the forgoing resume it became very clearly evident that treatment of ghee with 0.3 per cent curry leaves was most effective for reducing the peroxide formation in ghee during storage 80°±2°C, compared to the ghee with curry leaves at 0.1, 0.2 and 0.4 per cent rate. Any deviation from 0.3 per cent resulted in to decrease in performance of the treatment. Therefore, in the present study 0.3 per cent curry leaves was considered as optimum for treatment of ghee for better retention of flavour during storage.

Patel and Rajorhia (1979) [30] carried out the study dealing with the antioxidative effects of curry leaves (*Murraya koenigi*) when added to melted butter during clarification then heated to 120°C till characteristic ghee flavour developed. The amount of curry leaves added were 0.5, 0.8 and 1 % (w/v). Ghee samples were packed, sealed in lacquered tins and stored at 30°C. The antioxidative effect of various treatments in increasing order was exhibited by 0.5 % curry leaves, 0.8 % curry leaves and 1 % curry leaves. The peroxide values of ghee samples treated with curry leaves changed very little up to 30 days of storage. The control (without antioxidants) showed a steep rise in peroxide with value after 60 days of storage. Ghee samples added 1.0 per cent curry leaves showed least increase in peroxide value up to 135 days. The above cited report (Patel and Rajorhia, 1979) [30] is the only record available in the literature for application of curry leaves in treatment of ghee at different rates to evaluate its antioxidative effects in ghee. In that study addition of curry leaves at the rate of 1 per cent rate was found most effective, whereas, in present study 0.3 per cent was found most effective. Such variations might be attributed to number of reasons. There were several differences between work carried in present study and the study carried out by the authors to optimize rate of curry leaves: (1) form of curry leaves used (vacuum dried powder and fresh leaves respectively), (2) stage in ghee preparation at which curry leaves were added (final and initial respectively) and (3) temperature at which ghee was stored (80° and 30°C respectively). Even difference in variety might also be responsible for the difference, however, the authors did not reported the variety of the curry leaves used in their study.

Duga (1976) [34] pointed out that some antioxidants provide increased protection with increasing concentration, while others have optimal levels after which higher levels exert pro-oxidant effects. According to Fukumoto and Mazza (2000) [27] most phenolic compounds had pro-oxidant activity at low concentrations. Bouayed and Bohn (2010) [28] opined that high concentrations of antioxidants including BHT and BHA in food items, can also increase spoilage of food items due to pro-oxidant activities. Similar views were expressed by Ling *et al*. (2010) [35]. These authors stated that high concentrations of antioxidants may have pro-oxidant activity. Moure *et al*. (2001) [36] suggested that potent antioxidants can autoxidize and generate reactive substances and thus also act as pro-oxidants, depending on the systems, According to Gordon (1990) [33] as well as Cao and Cutler (1993) [37] at high concentrations of antioxidant, their pro-oxidant effects could arise due to the involvement of the phenolic compounds in initiation reactions (i.e., formation of radicals). As per Cillard *et al*. (1980) [38] hydroxyl radical absorbance capacity of antioxidants decreases at high concentrations due to their involvement in initiation reactions such as at high concentrations.

1.2 Changes in Flavour score of curry leaves treated ghee during storage at 80°±2°C

The results obtained for rate of curry leaves addition on

changes in flavour score of ghee during storage are presented in Table 4 and the trend is presented in Figure 4.

Table 4: Changes in flavour score of ghee during storage after treating with curry leaves at different rates

Storage period (days)	Flavour score of ghee during storage at 80°±2°C (Out of 9)				
	Rate of curry leaves used in treatment of ghee				
	0.0%	0.1%	0.2%	0.3%	0.4%
0	9.00	8.90	8.77	8.77	8.70
2	8.70	8.47	8.40	8.50	8.50
4	7.97	7.63	8.03	8.20	8.13
6	6.77	6.93	7.40	8.00	7.53
8	5.57	5.63	6.87	7.50	7.13
10	4.53	4.90	6.00	6.97	6.70
12	3.27	4.43	5.30	6.50	6.17
13	2.67	3.70	4.73	6.37	5.70
14	2.00	2.33	3.40	5.63	5.40
15	1.53	1.53	2.07	4.23	4.50
16	1.37	1.00	1.53	3.10	3.20
17	1.23	1.00	1.00	2.33	2.13
18	1.00	1.00	1.00	1.67	1.00
19	1.00	1.00	1.00	1.00	1.00
20	1.00	1.00	1.00	1.00	1.00
21	1.00	1.00	1.00	1.00	1.00
22	1.00	1.00	1.00	1.00	1.00

Source of Variation	Storage period (P)(days)	Treatment (T) (Rate of addition)	Interaction (P×T)
SEm	0.14	0.07	0.30
CD (0.05)	0.38	0.21	0.85
CV%	12.55		

The flavour score of different fresh ghee sample was in the order of control ghee > ghee treated with 0.1% curry leaves > ghee treated with 0.2% curry leaves = ghee treated with 0.3% curry leaves > ghee treated with 0.4% curry leaves. On 13th day of the storage the order of flavour score of different ghee samples was ghee treated with 0.3% curry leaves > ghee treated with 0.4% curry leaves > ghee treated with 0.2% curry leaves > ghee treated with 0.1% curry leaves > control ghee. However, at the end of the 22 days storage at 80°±2°C, flavour score of all the ghee samples were equal.

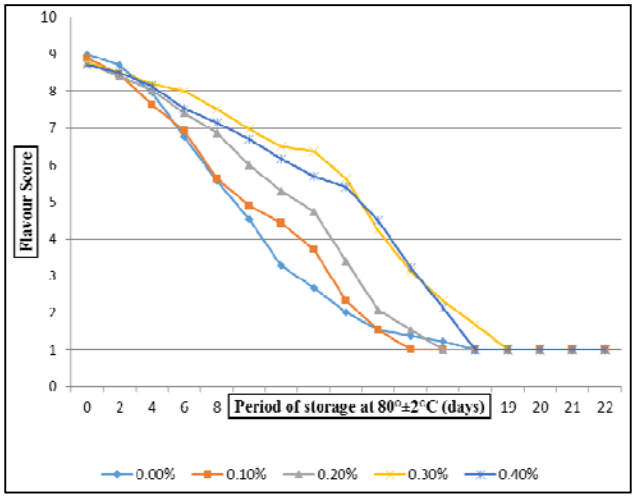


Fig 4: Changes in flavour score of ghee during storage after treating with curry leaves at different rates

It was revealed from statistical analysis that different rate of curry leaves used in treatment of ghee differed significantly ($P<0.05$) in their effect on changes in flavour score of ghee during storage. Similarly, storage period also differed significantly ($P<0.05$) in their effect on changes in flavour score of ghee. The interaction effect indicated that the rate of curry leaves used in treatment of ghee and storage period significantly differed from each other in their effect on flavour score of ghee over a storage period. Thus, it became evident that the effect of rate of curry leaves used for treatment of ghee and storage period were dependent on each other.

The flavour score of control ghee sample decreased at a rapid rate from beginning of the storage and showed steeped descale on 17th day of the storage. The sample of ghee treated with 0.1 per cent curry leaves also showed almost similar trend in changes in flavour score of ghee on storage as that of the control ghee sample. Interestingly, sample of ghee treated with 0.3 per cent curry leaves flavour score of ghee increased at a steady rate, no sharp rise in flavour score was observed in entire period of storage. The sample of ghee treated with 0.4 per cent curry leaves also showed almost similar trend in changes in flavour score of ghee on storage as that of the sample treated with 0.3 per cent curry leaves. However, trend in changes in flavour score of ghee treated with 0.2 per cent curry leaves was intermediate to that of the control ghee and the ghee treated with 0.1 per cent curry leaves.

Among the fresh ghee samples (0 day) only control sample of ghee acquired full flavour score (9 out of 9). All the samples of ghee treated with curry leaves at different rate of addition got lower flavour score compared control ghee sample and the decreased in the flavour score was proportional to the rate of curry leaves used for treatment of ghee. Thus, it was evident from the data that flavour score of the fresh ghee samples was decreasing with increasing rate of curry leaves used for treatment of ghee.

Among the samples of ghee treated with curry leaves at different rate of addition (0.1, 0.2, 0.3 and 0.4 per cent), the flavour score of ghee treated with 0.1 per cent curry leaves followed trend almost parallel to control ghee and with its values sometimes slightly lower and sometimes slightly higher than that of the control ghee sample. The flavour score of ghee treated with 0.2 and 0.4 per cent curry leaves followed trend almost parallel to the sample of ghee treated with 0.3 per cent curry leaves and with their values slightly lower than that of the ghee treated with 0.3 per cent curry leaves. The flavour score of ghee treated with 0.3 per cent curry leaves remained highest all throughout the storage.

Among all the samples of ghee, the flavour score of control ghee and ghee treated with 0.1 curry leaves went below the acceptable level on 8th day of the storage. On the other hand flavour score of ghee treated with 0.2, 0.3 and 0.4 per cent curry leaves went below the acceptable level on 12th, 14th and 13th day of the storage. Thus, among different levels of curry used in treatment of ghee, best control for retention of flavour score of ghee during storage was achieved by 0.3 per cent curry leaves. Any increase or decrease in the rate of curry leaves from 0.3 per cent resulted in to reduction in effectiveness of the treatment in controlling the deterioration ghee flavour during storage 80°±2°C. From the forgoing resume it became very clearly evident that treatment of ghee with 0.3 per cent curry leaves was most effective for reducing the deterioration of flavour of ghee during storage 80°±2°C.

Curry leaves contains phytochemical such as flavonoid, phenols, saponins, alkaloids and tannins. Flavonoids are the major constituent of curry leaves. The major constituent responsible for the aroma and flavour has been reported as pinene, sabinene, caryophyllene, cadinol and cadinene (Singh

et al., 2014; Igara *et al.,* 2016) [39, 1]. Therefore, decrease in flavour score of ghee upon addition of curry leaves observed in the present study, up to 0.4 per cent may be attributed to above mentioned constituents.

Patel and Rajorhia (1979) [30] carried out the study dealing with the antioxidative effects of curry (*Murraya koenigii*) leaves when added to melted butter during clarification and then heated to 120°C till characteristic ghee flavour developed. The amount of curry leaves 0.2, 0.5 and 1 per cent (w/v). Ghee samples were packed, sealed in lacquered tins and stored at 30°C. All the samples of ghee were rated excellent at the beginning of the experiment. The flavour scores of ghee samples treated with curry leaves and antioxidants changed very little up to 30 days of storage. Initial flavour score of control ghee was 8.33 whereas, ghee treated with 0.5, 0.8 and 1.0 per cent curry leaves received flavour score of 8.5, 8.4 and 8.4 respectively. After storage for 147 days flavour score of control ghee was 2.16 whereas, ghee treated with 0.2, 0.5 and 1.0 per cent curry leaves received flavour score of 5.5, 6.1 and 6.5 respectively.

Patel and Rajorhia, 1979 [30] is the only record available for application of curry leaves in ghee at different rates to evaluate its antioxidative effects in ghee. In that study addition of curry leaves at the rate of 1 per cent rate was found most effective, whereas, in present study 0.3 per cent was found most effective. Such variations might be attributed to number of reasons. There were several differences between work carried in present study and the study carried out by the authors to optimize rate of curry leaves: (1) form of curry leaves used (vacuum dried powder and fresh leaves respectively), (2) stage in ghee preparation at which curry leaves were added (final and initial respectively) (3) temperature at which ghee was stored (80° and 30°C respectively). Even difference in variety might also be responsible for the difference, however, the authors did not reported the variety of the curry leaves used in their study.

Comparison of curry leaves with synthetic antioxidant (BHA)

After selecting the appropriate stage and optimum rate of curry leaves addition in preparation of ghee, last phase of the study work was carried out to compare the performance of the curry leaves with butylated hydroxyl anisole (BHA), a synthetic antioxidant permitted in ghee under FSSAI rules for reducing the oxidative deterioration of ghee during storage.

1. Effect of antioxidants on oxidative changes in ghee on storage at 80°C

For comparing the effect of curry leaves addition in ghee with BHA added ghee, oxidative changes in ghee samples during storage at 80°±2°C were analysed for peroxide value and flavour score at regular interval of 2 days for 22 days.

1.1 Effect of antioxidants on peroxide value of ghee during storage at 80°C

The results obtained for changes in peroxide value of ghee during storage at 80°±2°C are presented in Table 5 and the trend is presented in Figure 5.

Table 5: Changes in peroxide value of ghee during storage at 80°C after treating with different antioxidants

Storage period (days)	Peroxide value of ghee (meq of O_2 per kg fat)		
	Control	Antioxidants used in ghee	
		Curry leaves (0.3%)	BHA (0.02%)
0	0.26	0.39	0.07
2	1.15	0.96	0.26
4	1.90	1.65	0.43
6	2.19	2.33	0.81
8	3.85	2.78	1.08
10	5.66	3.04	1.56
12	6.68	4.36	2.70
14	9.81	7.72	4.70
16	12.87	11.05	6.01
18	22.74	8.79	9.25
20	38.03	14.50	10.53
22	33.68	30.05	12.39

Source of variation	Storage period (P) (days)	Treatment (T) (antioxidant)	Interaction (P×T)
SE m	0.20	0.10	0.35
CD (0.05)	0.57	0.29	0.99
CV%	7.92		

The peroxide value of different fresh ghee samples was in the order of BHA > control > curry leaves. However, after the storage at 80°±2°C for 22 days the order of peroxide value of ghee was control > curry leaves > BHA.

It was revealed from statistical analysis that different antioxidants (BHA and curry leaves) used in treatment of ghee differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee during storage. Similarly, period of storage also differed significantly ($P<0.05$) in their effect on changes in peroxide value of ghee. The interaction effect indicated that different antioxidants used in treatment of ghee as well as period of the storage differed significantly from each other in their effect on peroxide value of ghee over a period of storage. Thus, it became evident that the effect of different antioxidants used in treatment of ghee and period of storage were dependent on each other.

Among the fresh ghee samples (0 day) peroxide value of control ghee sample was statistically at par with sample of ghee treated with curry leaves and remain at par up to 6th day of storage then after became significantly ($P<0.05$) higher than on subsequent storage up to end of the storage. However peroxide value of control ghee sample was also statistically at par with sample of ghee treated with BHA when sample were fresh (0 day) but then peroxide value of BHA added ghee sample was significantly ($P<0.05$) lower than control ghee sample on subsequent storage up to end of the storage. Moreover, peroxide value of curry leaves added ghee sample remained significantly ($P<0.05$) higher than that of the BHA added ghee sample during the entire storage period.

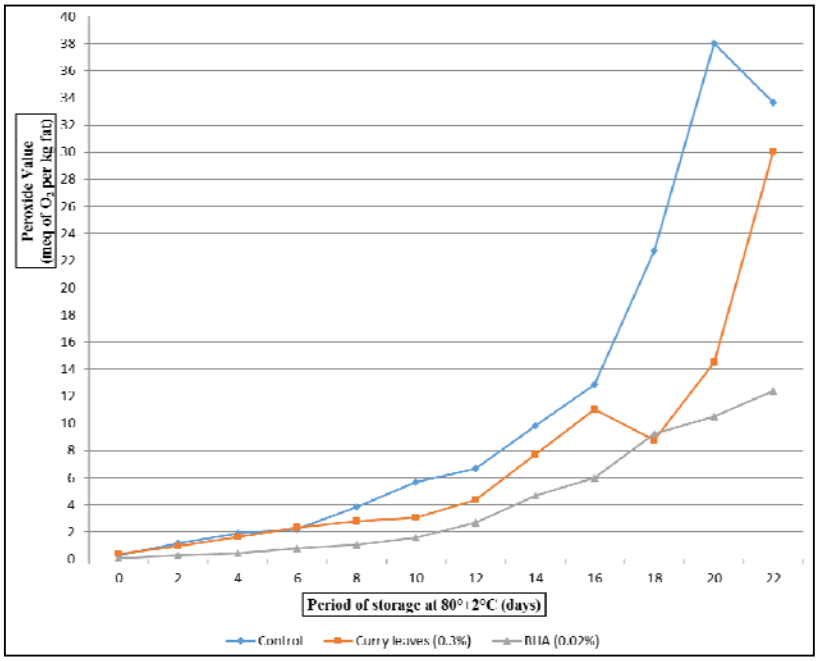


Fig 5: Changes in peroxide value of ghee during storage at 80°C after treating with different antioxidants

In control sample of peroxide value rise at very rapid from beginning, showed steeped rise in peroxide value on 14th day of the storage, reached to the maximum level (38.03 meq of O_2 per kg fat) on 20th days of the storage and then falling down on 22nd day of the storage. The ghee sample treated with curry leaves also followed almost similar trend of rise peroxide value up to 6th day of the storage as that of the control ghee. There was slight destine in peroxide value of this ghee sample ghee for a period of 4 days, then again it regain its moment of rise in the peroxide value, reached to the maximum level (30.05 meq of O_2 per kg fat) on 22nd day. In sample of ghee treated with BHA peroxide value rise very gradually up to 10th day, on further storage its rate of rise in peroxide value was accelerated, much steeped rise in peroxide value was noticed from 14th day of the storage and peroxide value reached to 12.39 meq of O_2 per kg fat on 22nd day of the storage.

On the basis of above findings, it can be inferred that addition of curry leaves exerts resistance towards the oxidative deterioration of ghee. From survey of literature it appears that no work is reported so far dealing with evaluation for relative effectiveness of BHA and curry leaves in controlling formation of peroxides in ghee during storage at 80°±2°C. Therefore, results obtained in the present study could not be compared as such with the reports in the literature. Pawar *et al.* (2012) [40] compared the potential of *Asparagus racemosus* (shatavari) extract with synthetic (butylated hydroxyanisole and tert-butyl hydroquinone) antioxidants, in ghee using accelerated oxidation tests. The aqueous and ethanolic extracts of shatavari were found to be less effective than the synthetic antioxidants.

4.6.3.4 Effect of antioxidants on flavour score of ghee during storage at 80°C

The results obtained for changes in flavour score of ghee during storage 80°±2°C are presented in Table 6 and the trend is presented in Figure 6.

Table 6: Changes in flavour score of ghee during storage at 80°C after treating with different antioxidants

Storage period (days)	Flavour score of ghee during storage at 80°±2°C (Out of 9)		
	Control	Antioxidants used in ghee	
		Curry leaves (0.3%)	BHA (0.02%)
0	9.00	8.57	9.00
2	8.77	8.10	9.00
4	8.27	7.63	9.00
6	7.30	7.40	8.60
8	6.60	7.00	8.20
10	5.77	6.73	7.67
12	4.90	6.40	6.90
14	2.70	4.80	5.27
16	1.33	2.90	4.07
18	1.00	2.07	3.00
20	1.00	1.93	2.57
22	1.00	1.53	2.00

Source of variation	Storage period (P) (days)	Treatment (T) (antioxidant)	Interaction (P×T)
SEm	0.27	0.13	0.46
CD (0.05)	0.76	0.38	NS
CV%	14.64		

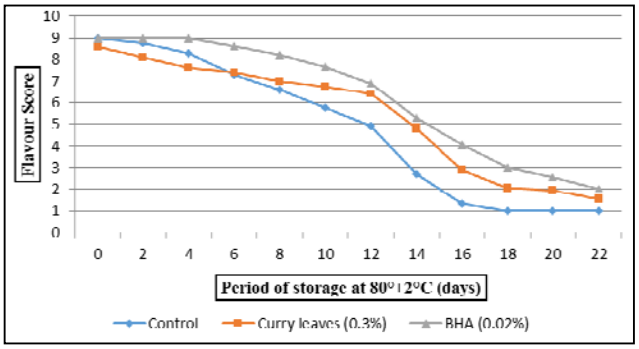


Fig 6: Changes in flavour score of ghee during storage at 80°C after treating with different antioxidants

The flavour score of different fresh ghee samples was in the order of control = BHA > curry leaves. However, after the storage at 80°±2°C for 22 days the order of flavour score of ghee was BHA> curry leaves > control. Among all the fresh ghee samples control ghee and the ghee added with BHA received full flavour score (9 out of 9). In control ghee sample flavour score decreased at slower rate from beginning, decline in flavour score was steeped from 4th day of the storage, it went below the acceptable level on 10th day and then went down on further storage. The sample of ghee treated with curry leaves alone also followed almost similar trend of decline in flavour score during the storage, as that of the control sample of ghee, but comparatively at slightly lower rate than the control. The flavour score of curry leaves treated ghee sample went below the acceptable level on 14th day and continued to decline on further storage. In sample of ghee treated with BHA flavour score remain constant up to first 4 days of the storage and remained quite above the flavour score of sample of ghee treated with curry leaves. However, on further storage flavour score of the BHA added ghee sample started declining at rapid rate and sharp decline in flavour score was noticed from 10th day of the storage. The flavour score of BHA treated ghee sample went below the acceptable level on 14th day of the storage and continued to decline on further storage.

It was revealed from statistical analysis of data that the treatments (BHA and curry leaves used in ghee as antioxidants) and storage period both were significant ($P<0.05$) on changes in flavour score of ghee during storage. The interaction between storage period as well as antioxidants used in ghee was statistically non-significant. Thus, results revealed that treatments and storage period have significant effect on changes in flavour score of ghee during storage. However, the interaction effect of storage period and treatments was non-significant.

Flavour score of control ghee sample remained significantly ($P<0.05$) lower than that of the curry leaves added ghee sample during the entire storage period except on 6th day. On 6th day flavour score of control ghee sample was statistically at par with curry leaves added ghee. However, flavour score of control ghee was statistically at par with BHA added ghee sample up to 2 days but then after the flavour score of BHA added ghee sample was remained significantly ($P<0.05$) higher than that of the control ghee. Moreover, flavour score of BHA added ghee sample remained significantly ($P<0.05$) higher than that of the curry leaves added ghee sample during the entire storage period. From survey of literature it appears that no work is reported with evaluation for relative effectiveness of BHA and curry leaves in controlling flavour deterioration in ghee during storage at 80°±2°C. Therefore, results obtained in the present study could not be compared as such with the reports in the literature.

Summary and Conclusion

The present study was conducted to evaluate potential of curry leaves (*Murraya koenigii*) as an antioxidant in ghee to extend the shelf-life by retarding oxidative reactions during its storage. The total phenolic content and radical scavenging activity of curry leaves evaluated in the study was within the range reported in the literature. For addition of curry leaves in treatment of ghee final stage of heat clarification found more effective than the initial stage of heat clarification. Treatment of ghee with different rate of curry leaves (0.1%, 0.2%, 0.3% and 0.4%), the rate 0.3 per cent was found most effective in reducing peroxide formation and flavour deterioration in ghee during storage. Curry leaves was found to be capable of retarding oxidative degradation in ghee but were less effective than BHA. Hence, curry leaves could be used as a natural antioxidant to preserve the food system apart from providing other beneficial benefits and would be preferred over BHA to minimize adverse effects on mankind.

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