International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 6 Issue 7, November-December 2022 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Evaluation of Betel Leaves (*Piper Betel*) for Enhancing Shelf-Life of Ghee (Heat Clarified Milk Fat) against Oxidative Deterioration

Kapadiya Dhartiben B., Aparnathi K. D.

Dairy Chemistry Department, SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat, India

ABSTRACT

Betel leaves was evaluated as an antioxidant to enhance the shelf-life of ghee (heat clarified milk fat) against oxidative deterioration. Addition of betel leaves at final stage of heat clarification was found more effective in controlling oxidative deterioration of the ghee than that of the initial stage of heat clarification. The optimum rate for use of betel leaves in treatment of ghee was found 0.3%. The addition of betel leaves @ 0.3% of the expected yield of ghee at the final stage of the heat clarification in preparation of ghee found highly effective in retarding its oxidative deterioration during storage. It was even more effective than most popular synthetic antioxidant BHA.

KEYWORDS: *betel leaves, oxidative deterioration, ghee*

How to cite this paper: Kapadiya Dhartiben B. | Aparnathi K. D. "Evaluation of Betel Leaves (Piper Betel) for Enhancing Shelf-Life of Ghee (Heat Clarified Milk Fat) against Oxidative Deterioration" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-6 | Issue-7, December 2022, pp.470-484, URL: www.ijtsrd.com/papers/ijtsrd52355.pdf

IJTSRD52355

1. INTRODUCTION

India is well-known throughout the world for its medicinal plants and spices. Both have many different physiological and pharmacological characteristics. The current biomedical research is concentrated on their scientific merits, to generate functional foods or nutraceuticals, to give science-based proof for the traditional usage, and to do so. With more thorough research into natural medicines over the past 10 years, plants have long been a vital source of natural ingredients to sustain human health.

Due to their potential for providing nutrition, safety, and medicinal benefits, herbal foods have garnered significant interest in recent years. The need for herbal meals is rising as a result of public interest and consumer demand, as well as ongoing research efforts to identify the characteristics, bioactive components, and prospective applications. Due to its safety, its use in a variety of fields of study, including pharmaceutics, ayurveda, and the food sector, is expanding.

Ghee oxidatively degrades while being stored in ambient conditions. Synthetic antioxidants are frequently added to ghee to extend its shelf life, but their use has drawn attention away from natural antioxidants because they may pose a health risk. Although there has been very little research on the use of herbs as potential antioxidants in ghee, they are thought to be very good sources of natural antioxidants. There have been some attempts to test the effects of herbal extracts in organic solvents in ghee.

However, using such extracts may give the product a very unpleasant aroma. Furthermore, since some minor components that are important to the activity due to their synergistic effect may be lost during the preparation of the extract, extracts are less effective in their action compared to whole herbs (Shahidi, 2015; Kapadiya et al., 2016). Another restriction is the existence of residual organic solvents in the extract (Pawar et al., 2012; Patel et al., 2013).

In my previous work common herbs used in routine diet were screened for their total phenolic content, radical scavenging activity, compatibility in ghee from sensory point of view and possibility to act as an antioxidant in ghee. Among the fourteen different herbs, betel leaves (*Piper betle*) was found most promising (Kapadiya and Aparnathi, 2018). Curry leaves was found to be capable of retarding oxidative degradation in ghee but were less effective than BHA (Kapadiya and Aparnathi, 2017). Liquorice was found to be capable of retarding oxidative degradation in ghee and even more effective than BHA (Kapadiya and Aparnathi, 2017).

The piper betle is a well-liked plant in South Asia and is known as the "Green Gold of India." It is a perennial herb (Kumari and Rao, 2015). It was used as a traditional medicine in South East Asia, China, and India (Chiang Chan and Wong, 2014). Various biologically active substances can be found in betel leaves (Nirmala and Kumari, 2015). Polyphenols like eugenol, chavicol, charvacrol, chevibetol, catechol, and allyl pyrocatechol, which have strong antioxidant activity, were discovered to be the primary chemical components of Piper betel (Chauhan et al., 2016).

Therefore, the present study was planned to evaluate the potential of dried betel leaves for enhancing shelf-life of ghee against oxidative deterioration. The study was divided into three phases: selection of stage for addition, optimization of rate for addition and comparison with synthetic antioxidant (BHA).

2. Materials and methods

2.1. 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.

2.2. Collection and preparation of betel leaves

Betel leaves (*Piper betle*) were obtained from Medicinal and Aromatic Plants Research Center of the Anand Agricultural University. Leaves were cleaned, vacuum dried, grinded to coarse particles, transferred into zip lock plastic cover and stored in air tight plastic bottle at refrigeration temperature. Fresh and dried betel leaves are shown in Figure 1.

Fresh betel leaves	Dried betel leaves

Figure 1: Fresh and dried betel leaves

2.3. Evaluation of betel leaves for its antioxidant potential

2.3.1. Preparation of extract

0.5 g powder of betel 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). The mixture was then centrifuged at 4,000 rpm for 10 min. The supernatant (betel leaves extract) was recovered for the determination of the total phenolic content and radical scavenging activity.

2.3.2. Analysis of total phenolic content

Total phenolic content of betel leaves extract was analysed by Folin-Ciocalteu (FC) reagent according to the procedure described by Singleton and Rossi (1965).

0.03 ml betel 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 content was mixed using vortex mixer and 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 betel leaves.

2.3.3. Radical scavenging activity by DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) assay

The radical-scavenging activity of betel leaves extract was determined as the ability to scavenge DPPH radicals according to the procedure of Brand-Williams *et al*. (1995).

0.05 ml of betel 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 tubeand shaken vigorously. The tube was allowed to stand at 37°C for 15 minutes 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 a blank. Absorbance of the control and sample was 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

2.4. Preparation of ghee

Ghee was prepared by creamery butter method as described by De (2004). White butter was procured from commercial dairy plant, clarified at 120°C temperature, followed by filtration through 6 layered muslin cloth.

2.5. Selection of stage for addition

Betel leaves was added @ 0.5% during manufacture of ghee at initial stage of the heat clarification (*i.e.* when temperature reached 45°C) and at final stage of the heat clarification (*i.e.* when temperature reached 105°C). The heating continued till temperature reached to 120°C. The treated ghee samples were filtered through 6 layers of muslin cloth. Ghee without betel leaves was also prepared simultaneously to serve as a control. All the ghee samples were tested for stability against oxidative deterioration during storage at 80°±2°C and monitored for changes in peroxide value and flavour score at an interval of 2 days till peroxide values of all the ghee samples went below acceptable level (< 6). The stage of addition giving better stability was selected for further study.

2.6. Optimization of rate for addition

Betel leaves was added @ 0.0 (control), 0.1, 0.2, 0.3 and 0.4% of the expected yield of ghee at the stage in heat clarification found better in the study described under Section 2.4. All the ghee samples were stored in an incubator at 80°±2°C and monitored for changes in peroxide value and flavour score at an interval of 2 days till peroxide values of most of the ghee samples went below acceptable level (< 6). The rate of addition giving the best stability was selected for further study.

2.7. Comparison with synthetic BHA

Ghee sample was prepared with betel leaves at the selected stage and optimized rate. Simultaneously, two samples of ghee were prepared without betel leaves. In one of these samples BHA was added @ 0.02% (w/w) and other was kept as control. All the ghee samples were stored at 80°±2°C temperature. Oxidative changes taking place in ghee samples were analysed for peroxide value, carbonyl value, DPPH radical scavenging activity and flavour score. Ghee samples were monitored when fresh and after an interval of 2 days at 80°±2°C till flavour score of all the ghee samples went below acceptable level (< 6).

2.8. Determination of oxidative changes in ghee

The peroxide value of ghee was determined by iodometric method as described in IS: SP: 18 (part XI) (1981). The carbonyl value of ghee was determined as per the method of Ronald (2001). Sensory evaluation for flavour score was evaluated using the 9-point hedonic scale (Mehta et al., 2015). Data were analysed by completely randomized design at 5% level of significance ($p<0.05$) as per the procedure mentioned by Steel and Torrie (1980).

3. Results and discussion

3.1. Assessing the compatibility of betel leaves as an additive in ghee

The first and foremost point for consideration was the compatibility of betel leaves to be used for study. Betel leaves was added at the rate of 0.5% w/w of ghee and then the compatibility of betel leaves added ghee sample was assessed based on their flavour. Colour characteristic of betel leaves added ghee sample was also examined by visual observation. The results indicated that betel leaves treated ghee was found acceptable for their flavour. The colour characteristic of the fresh ghee samples (control ghee and betel leaves added ghee) is presented in Figure 2.

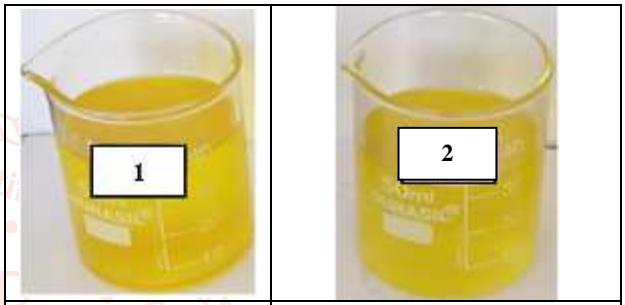

Figure 2: The colour characteristic of the fresh ghee samples

It was evident from the examination of the colour characteristic of ghee samples that both the ghee samples acquired golden yellow colour. Colour of betel leaves did not impart any objectionable colour to the ghee.

3.2. Evaluation of betel leaves for its antioxidant potential

To evaluate the antioxidant potential, betel leaves was analysed for its total phenolic content and radical scavenging activity. Total three replication were conducted. Betel leaves gave 3653.33 ± 18.86 mg GAE/100 g total phenolic content and 87.31 ± 0.77% DPPH radical scavenging activity.

Jamal *et al.* (2010) studied forty types of Malaysian medicinal plants and found that *Piper betel* L. had phenolic content of 8986.67 mg per liter GAE. Jaiswal *et al.* (2014) collected six different variety of betel leaf [Banarasisafeda (PA), Calcutta (PB), Cuttack (PC), Desibagla (PD), Maharashtra (PE) and Sofia (PF)] from Uttar Pradesh, West Bengal, Odisha, Maharashtra and Bihar and were extracted using five solvents (80% methanol, 80% ethanol, 80% acetone, 80% ethyl acetate and distilled water) to determine total phenol content and radical scavenging activity. The TPC for six variety betel leaf extract in five different solvents were found in the range of 0.29 to

2.62 mg GAE per g dw for PA, 0.08 to 2.59 mg GAE per g dw for PB, 0.09 to 2.38 mg GAE per g dw for PC, 0.07 to 2.87 mg GAE per g dw for PD, 0.16 to 2.07 mg GAE per g dw for PE and 0.04 to 1.16 mg GAE per g dw for PF.

The total phenolics content and radical scavenging activity of betel leaves evaluated in the study was more or less 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), prevailing agroclimatic conditions of the area in which herb is grown (Jaiswal *et al.,* 2014), agronomic practises followed in the plant farming (Pakade *et al.,* 2013), maturity of the herb at the stage of harvesting (Jinesh *et al.,* 2010), method followed for post-harvest processing of the herb (Pakade *et al.,* 2013), the type and concentration of solvent (Hasim *et al.*, 2015) as well as polarity of solvent (Basak *et al.*, 2014) used for analysis, the method followed for the estimation of the total phenolics content (Rahman *et al.*, 2013), etc.

However, some deviations in radical scavenging activity might be attributed to variations in chemical composition of the different plants with respect to phenolics and other antioxidants as well as pro-oxidants content (Tupe *et al.,* 2013).

3.3. Selection of stage for add betel leaves in preparation of ghee

Based on procedure for production of ghee, two different stages can be evinced for addition of betel leaves: initial stage and final stage of the heat clarification process. When betel leaves was added at initial stage of the heat clarification, it remains in contact with ghee for longer duration and leads to better extraction of antioxidants. However, longer exposure at elevated temperature may adversely affect the stability of major and/or minor antioxidant components, leading to decrease in effectiveness. Therefore, it was decided to find appropriate stage for addition of betel leaves in preparation of ghee.

Changes in peroxide value on storage of the ghee samples treated with betel leaves at two different stages are presented in Table 1 and the trend is graphically presented in Figure 3(a). Similarly, changes in flavour score on storage of the ghee samples treated with betel leaves at two different stages are presented in Table 2 and the trend is graphically presented in Figure 3(b).

Table 1: Effect of stage for addition of betel leaves on changes in peroxide value of ghee during storage at 80°±2°C

Storage period (days)	Peroxide value of ghee on storage at 80°±2°C (meq of O_2/kg fat)		
	Control	Betel leaves added at initial stage of clarification	Betel leaves added at final stage of clarification
0	0.19	0.63	0.60
2	0.53	1.50	1.28
4	1.44	2.31	1.62
6	1.93	3.07	2.46
8	4.04	4.23	2.81
10	6.19	5.43	3.72
12	9.33	7.09	5.05
Source of variation	**Storage period (P) (Days)**	**Treatment (T) (Stage of addition)**	**Interaction (P×T)**
SEm	0.18	0.12	0.31
CD(0.05)	0.51	0.34	0.89
CV%	20.19		

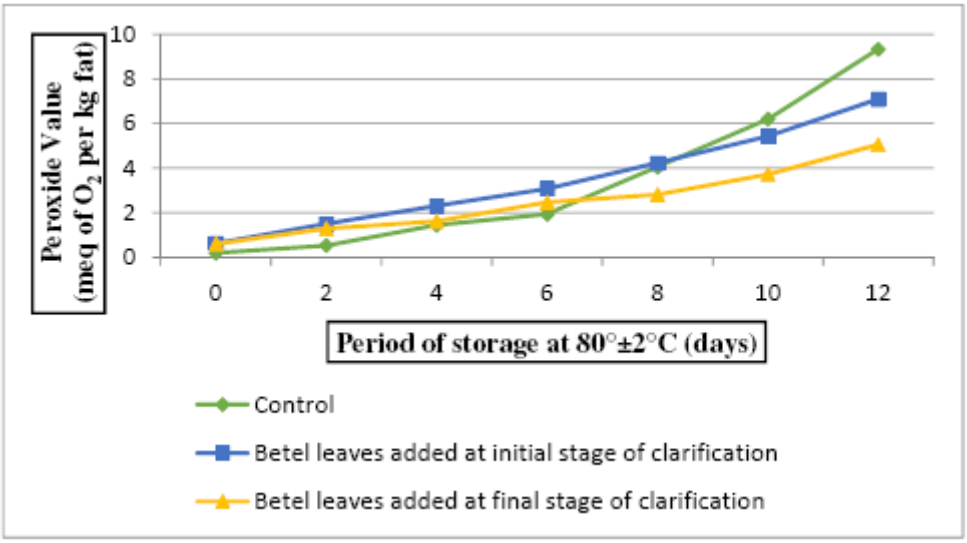

Figure 3(a): Changes in peroxide value of betel leaves treated ghee during storage

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

After 2nd day of the storage peroxide values of ghee sample treated with betel leaves at initial stage of clarification were significantly higher than that of ghee sample treated with betel leaves at final stage of clarification.

Table 2: Effect of stage for addition of betel leaves on changes in flavour score of ghee during storage at 80°±2°C

Storage period (days)	Flavour score of ghee during storage at 80°±2°C (out of 9)		
	Control	Betel leaves added at initial stage of clarification	Betel leaves added at final stage of clarification
0	9.00	8.80	8.83
2	8.58	8.30	8.33
4	7.75	7.88	7.90
6	6.60	7.48	7.53
8	5.60	7.05	7.15
10	4.10	6.68	6.70
12	2.40	6.20	6.35
Source of variation	**Storage period (P) (Days)**	**Treatment (T) (Stage of addition)**	**Interaction (P×T)**
SEm	0.04	0.03	0.07
CD (0.05)	0.12	0.08	0.21
CV%	2.11		

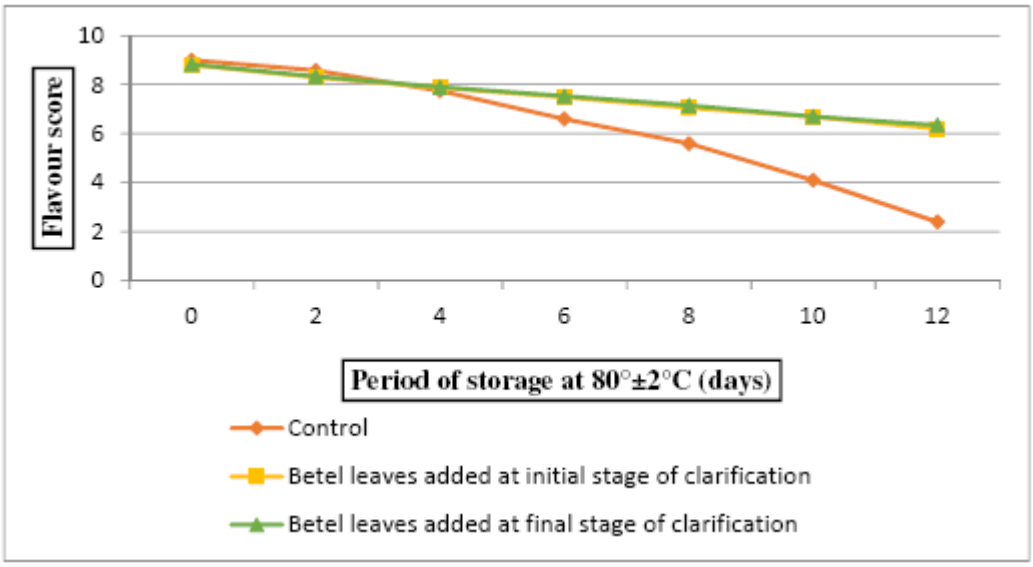

Figure 3(b): Changes in flavour score of betel leaves treated ghee during storage

The flavour score of all the three types of ghee samples declined at a steady rate up 6th day of the storage. On subsequent storage in control ghee and ghee treated with betel leaves at initial stage of clarification, the rate of decline in flavour score became sharp. On the other hand in ghee treated with betel leaves at final stage of clarification, no sharp decline in flavour score was noticed during the entire storage period. At the end of the storage the flavour score of ghee treated with betel leaves at final stage of clarification was significantly higher than the ghee treated with betel leaves at initial stage of clarification.

Thus, it was evident that the addition of betel leaves at the final stage of heat clarification in preparation of ghee was significantly more efficient in controlling oxidative deterioration of ghee during storage, compared to the addition of betel leaves at initial stage of heat clarification. No report is available in the literature effect of stage for addition of betel leaves on changes in peroxide value of ghee during storage. However, similar work carried by Kapadiya and Aparnathi (2017) on evaluation of curry leaves (*Murraya koenigii*) for enhancing shelf-life of ghee against oxidative deterioration, found that the addition of curry leaves at final stage of heat clarification were found more effective than at initial stage of heat clarification. Thus, results obtained in present study were in accordance with the results reported by this author.

Kim et al. (2006) studied the effect of heat on the antioxidant capacity of grape seed extracts and concluded that the antioxidant capacity of these extracts increased through the liberation of phenolic compounds by heat. According to Nicoliy et al. (1999) polyphenols with an intermediate oxidation state can exhibit higher radical scavenging efficiency than the non-oxidized ones. The higher antioxidant properties of the partially oxidized polyphenols could be attributed to their increased ability to donate a hydrogen atom from the aromatic hydroxyl group to a free radical and/or to the capacity of their aromatic structures to support the unpaired electron through delocalization around the π-electron system. Therefore, in present study better performance of betel leaves used at final stage of clarification during preparation of ghee compared to an initial stage of the clarification 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 by various authors.

3.4. Optimization of rate for addition of betel leaves in preparation of ghee

The effect of antioxidant depends on its concentration, both at low and high concentration they may become pro-oxidant (Gordon, 1990). Moreover, aroma associated with herb alters typical aroma of ghee to some extent. Therefore, work was carried out to optimize rate of betel leaves for the use. Changes in peroxide value of ghee during storage at 80°±2°C after treating with betel leaves at different rates are presented in Table 3 and the trend is presented in Figure 4(a). Similarly, changes in flavour score of ghee during storage at 80°±2°C after treating with betel leaves at different rates are presented in Table 4 and the trend is graphically presented in Figure 4(b).

Table 3: Effect of different rates of betel leaves on changes in peroxide value of ghee during storage at 80°±2°C

Storage period (days)	Peroxide value of ghee on storage at 80°±2°C (meq of O_2/kg fat)				
	Rate of betel leaves used (%)				
	0.0	0.1	0.2	0.3	0.4
0	0.19	0.39	0.32	0.19	0.26
2	0.77	0.90	0.78	0.58	0.64
4	1.67	1.69	1.48	1.17	1.16
6	3.68	1.80	1.85	1.20	1.24
8	4.73	3.61	2.41	2.18	2.18
10	6.71	4.39	2.76	2.35	2.41
12	8.71	4.76	3.19	2.53	2.54
13	10.88	5.01	3.56	2.66	2.82
14	12.79	5.41	4.17	2.71	3.28
15	14.72	5.91	5.34	2.75	3.65
16	17.41	7.41	6.64	2.81	4.03
17	20.35	8.91	7.09	2.87	4.33
18	24.71	10.44	7.57	2.92	5.38
19	27.76	11.71	11.31	2.97	6.39
20	31.02	13.03	13.32	3.12	7.57
21	32.97	15.56	14.89	3.31	9.48
22	35.80	18.95	17.16	3.51	11.91

Source of variation	Storage period (P) (Days)	Treatment (T) (Rate of addition)	Interaction (P×T)
SEm	0.25	0.13	0.55
CD (0.05)	0.69	0.37	1.53
CV%	13.79		

Among the fresh ghee samples control ghee had lower peroxide value compared to ghee samples treated with betel leaves at different rate of addition except 0.3 per cent treated ghee sample. The peroxide value of control ghee became significantly (P<0.05) higher than that of betel leaves treated ghee samples from 6th day of storage and on subsequent storage up to end it remained significantly (P<0.05) higher than that of betel leaves treated ghee samples.

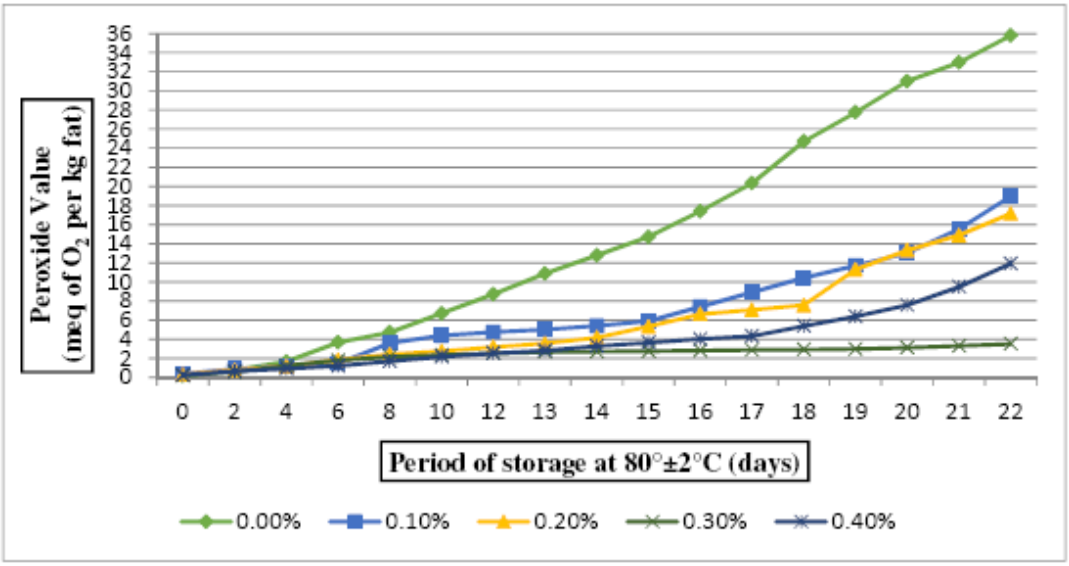

Figure 4(a): Changes in peroxide value of ghee during storage after treating with betel leaves at different rates

Among the betel leaves treated ghee samples at different rate of addition, peroxide values of all the betel leaves treated ghee samples were statistically at par up to 4th day of the storage. On further storage peroxide values of ghee sample treated with 0.3% betel leaves was significantly (P<0.05) lower than the ghee samples treated with 0.1 and 0.2% betel leaves. Similarly, after 13th day of storage period peroxide value of ghee sample treated with 0.3% cent betel leaves was significantly (P<0.05) lower than 0.4% betel leaves treated ghee sample. It can be sum-up that treatment of ghee with betel leaves at different rates, best control of peroxide formation in ghee during storage was given by the treatment @ 0.3%. Therefore, 0.3% betel leaves was considered as optimum and selected for further study.

Patel and Rajorhia (1979) studied the antioxidative effects of betel leaves in ghee. Fresh betel leaves were added to different lots of melted butter and then heated to clarification temperature (120°C). The betel leaves were added 0.2, 0.5 and 1% (w/v). Ghee samples were packed, sealed in lacquered tins and stored at 30°C. The authors reported that the control ghee showed a steep rise in peroxide with value after 60 days of storage. However, ghee samples treated with betel leaves 1 per cent concentration proved to be most stable even up to 147 days of storage at 30°C. These authors used fresh betel leaves and ghee samples were stored at 30°C, whereas, in the present study dried betel leaves was used and samples were stored at 80°C.

Table 4: Effect of different rates of betel leaves on changes in flavour score of ghee during storage at 80°±2°C

Storage period (days)	Flavour score of ghee during storage at 80°±2°C (Out of 9)				
	Rate of betel leaves used in the treatment				
	0.0%	0.1%	0.2%	0.3%	0.4%
0	9.00	9.00	9.00	8.90	8.73
2	8.70	8.67	8.73	8.83	8.50
4	7.97	8.07	8.23	8.73	8.23
6	6.77	7.33	8.50	8.00	7.63
8	5.57	6.27	8.23	7.70	7.40
10	4.53	5.40	6.93	7.47	7.23
12	3.27	4.80	6.27	7.27	6.93
13	2.67	4.23	6.00	7.00	6.77
14	2.00	3.73	5.43	6.80	6.63
15	1.53	3.53	5.17	6.67	6.23
16	1.37	2.97	4.93	6.47	5.93
17	1.23	2.33	4.47	6.20	5.73
18	1.00	1.93	4.17	6.03	5.47
19	1.00	1.20	3.73	6.00	5.27
20	1.00	1.00	2.93	6.00	4.53
21	1.00	1.00	2.53	6.00	3.83
22	1.00	1.00	2.00	6.00	3.00

Source of Variation	Storage period (P) (Days)	Treatment (T) (Rate of addition)	Interaction (P×T)
SEm	0.08	0.05	0.19
CD (0.05)	0.24	0.13	0.53
CV%	6.09		

The flavour score of fresh ghee samples treated with 0.3 and 0.4% betel leaves was lower than other ghee samples. The typical aromatic flavour of betel leaves is due to presence of phenol and terpene-like compounds (Uddin et al., 2015; Nirmala and Kumari, 2015). Therefore, decrease in flavour score of fresh ghee with 0.3 and 0.4% betel leaves may be attributed to such constituents of betel leaves.

The flavour score of control ghee decreased at a rapid rate from beginning of the storage and showed steep descale on 17th day. Among the samples of ghee treated with betel leaves at different rate of addition, the flavour score of ghee treated with 0.1 per cent betel leaves followed trend almost parallel to control ghee and with its values slightly higher than that of the control ghee. The flavour score of ghee treated with 0.2 and 0.4 per cent betel leaves followed trend almost parallel to each other and the values of 0.2 per cent betel leaves treated ghee slightly lower than that of the 0.4 per cent betel leaves treated ghee. The flavour score of ghee treated with 0.1 per cent betel leaves remained lowest and 0.3 per cent betel leaves remained highest all throughout the storage.

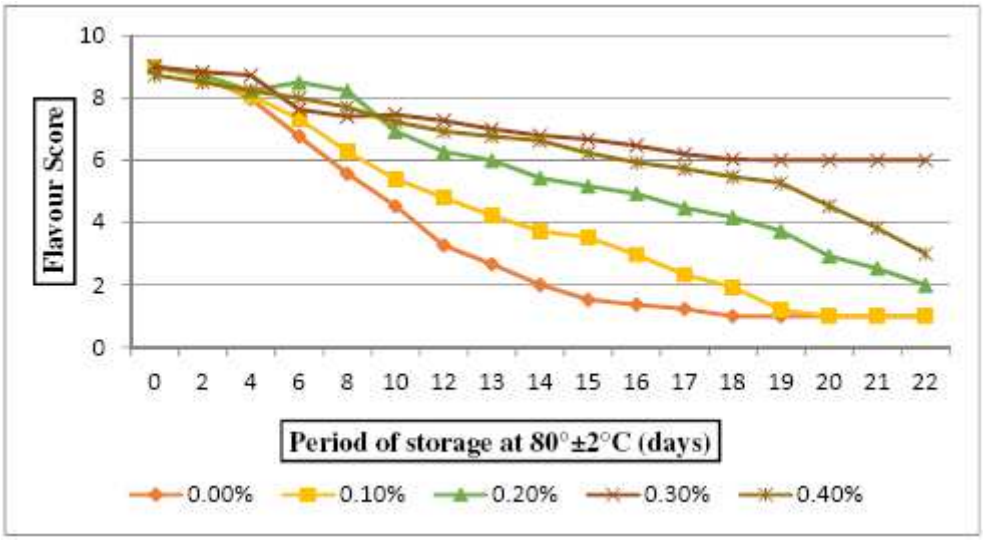

Figure 4(b): Changes in flavour score of ghee during storage after treating with betel leaves at different rates

Flavour score of control ghee went below the acceptable level on 8th day of the storage that of the ghee treated with 0.1, 0.2 and 0.4% betel leaves on 10th, 14th and 16th day respectively. However, flavour score of ghee treated with 0.3% per cent betel leaves was acceptable event on 22nd day. It is clearly evident from the results that treatment of ghee with 0.3% betel leaves was most effective in retarding flavour deterioration of ghee during the storage.

The variation in effectiveness of betel leaves in controlling oxidative deterioration of ghee during storage with variation in its rate of use may be attributed difference in its behaviour at low and high concentration. The low effectiveness of betel leaves at 0.1 and 0.2% might be attributed insufficient antioxidant principles available for action and/or their possible pro-oxidant effect at lower concentration. Similarly, low effectiveness of betel leaves at 0.4% might be attributed possible pro-oxidant effect due to presence in higher concentration. Duga (1976) 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) most phenolic compounds had pro-oxidant activity at low concentrations. Moure et al. (2001) suggested that potent antioxidants can autoxidize and generate reactive substances and thus also act as pro-oxidants, depending on the systems. According to Bouayed and Bohn (2010) at high concentrations of antioxidant, their pro-oxidant effects could arise due to the involvement of the phenolic compounds in initiation reactions.

3.5. Comparison of betel leaves with synthetic antioxidant BHA

After selecting appropriate stage for addition of betel leaves and optimizing rate of the addition, work was carried out to compare its effect with most popular synthetic antioxidant (BHA). In process of lipid oxidation, during primary stage of the oxidation peroxides are formed. The peroxides are unstable and generate secondary oxidation products including aldehydes, ketones, epoxides, etc. These compounds are major contributors to off-flavours developed due to oxidative rancidity of many foods (Mehta et al., 2015). Therefore, in final phase of this study oxidative changes in ghee samples during storage were monitored by changes in peroxide value, carbonyl value, radical scavenging activity and flavour score of the ghee samples during storage at 80°±2°C till flavour score of almost all the ghee samples went below the acceptable level (< 6). The results obtained for effect of antioxidants on changes in peroxide value and carbonyl value of ghee during storage at 80°±2°C are presented in Table 5 and trends are graphically presented in Figure 5(a) and Figure 5(b). The results obtained for effect of antioxidants on changes in radical scavenging activity and flavour score of ghee during storage at 80°±2°C are presented in Table 6 and trends are graphically presented in Figure 5(c) and Figure 5(d).

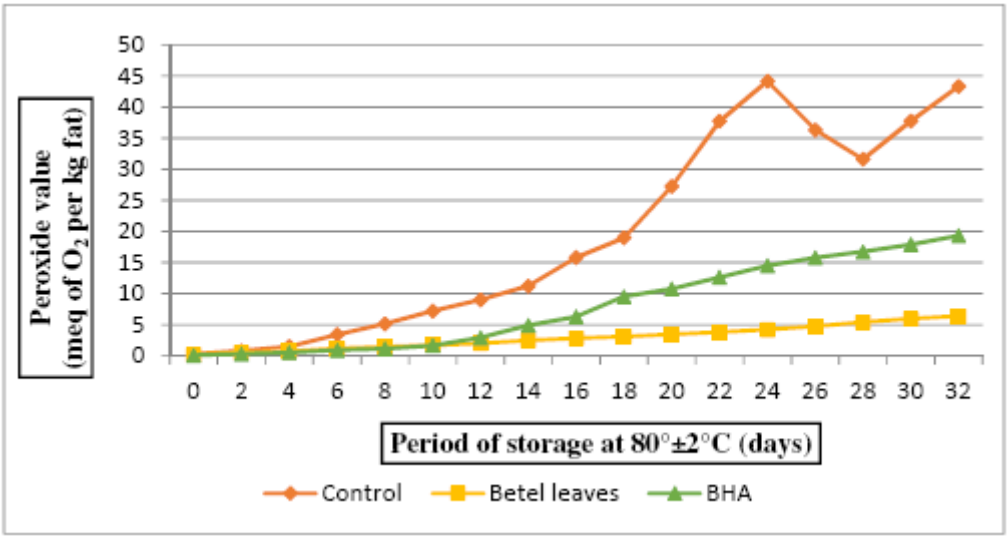

Figure 5(a): Changes in peroxide value of ghee during storage at 80°±2°C after treating with different antioxidants

Table 5: Effect of antioxidants on changes in peroxide value and carbonyl value of ghee during storage at 80°±2°C

Storage period (days)	Peroxide value of ghee on storage at 80°±2°C (meq of O_2/kg)			Carbonyl value of gheeon storage at 80°±2°C (µmol carbonyl/g)		
	Control	Antioxidants used in ghee		Control	Antioxidants used in ghee	
		Betel leaves (0.3%)	BHA (0.02%)		Betel leaves (0.3%)	BHA (0.02%)
0	0.19	0.29	0.10	3.25	3.37	3.36
2	0.82	0.53	0.29	3.92	3.68	3.64
4	1.52	0.74	0.52	4.42	3.89	3.98
6	3.43	1.17	0.91	5.74	4.16	4.34
8	5.15	1.46	1.16	7.81	4.56	4.88
10	7.20	1.71	1.66	8.20	5.04	5.36
12	9.00	2.03	2.93	10.05	5.67	5.95
14	11.22	2.49	4.92	10.84	6.14	6.59
16	15.81	2.84	6.30	12.32	6.96	7.58
18	18.98	3.10	9.53	14.74	7.51	8.50
20	27.26	3.44	10.79	16.43	7.95	9.50
22	37.76	3.83	12.64	18.72	8.64	10.31
24	44.17	4.22	14.51	21.93	9.05	11.25
26	36.34	4.73	15.78	25.92	9.46	12.51
28	31.62	5.43	16.74	29.66	9.91	13.37
30	37.75	5.96	17.90	34.23	10.49	14.94
32	43.36	6.38	19.36	36.00	11.29	16.61

Source of variation	Storage period (P) (Days)	Treatment (T) (Antioxidant)	Interaction (P×T)	Source of variation	Storage period (P) (Days)	Treatment (T) (Antioxidant)	Interaction (P×T)
SEm	0.42	0.18	0.73	SEm	0.10	0.04	0.18
CD (0.05)	1.18	0.50	2.05	CD (0.05)	0.29	0.12	0.50
CV%	14.43			CV%	3.47		

Table 6: Effect of different antioxidants on changes in radical scavenging activity and flavour score of ghee during storage at 80°±2°C

Storage period (days)	Radical scavenging activity of ghee (% inhibition)			Flavour score of ghee during storage at 80°±2°C(Out of 9)		
		Antioxidants used in ghee			Antioxidants used in ghee	
	Control	Betel leaves (0.3%)	BHA (0.02%)	Control	Betel leaves (0.3%)	BHA (0.02%)
0	43.36	61.98	61.35	9.00	8.82	9.00
2	42.06	60.68	59.14	8.60	8.58	9.00
4	40.99	57.92	57.11	8.13	8.28	9.00
6	38.44	56.06	54.35	6.98	8.10	8.50
8	34.85	54.27	52.46	5.45	7.90	8.15
10	32.87	52.63	49.71	4.85	7.75	7.55
12	29.53	49.19	47.12	4.38	7.63	6.73
14	26.10	47.55	44.24	3.35	7.43	5.25
16	23.92	44.08	41.86	2.90	7.33	4.13
18	21.52	41.38	37.75	1.88	7.18	2.93
20	18.11	39.01	35.54	1.43	7.03	2.43
22	14.44	35.19	31.75	1.25	6.83	1.88
24	12.21	31.49	26.97	1.05	6.75	1.65
26	9.93	28.52	23.20	1.00	6.33	1.33
28	7.69	25.89	20.01	1.00	6.10	1.25
30	5.94	23.84	16.71	1.00	6.00	1.25
32	4.60	21.77	12.10	1.00	5.43	1.13

Source of variation	Storage period (P) (Days)	Treatment (T) (Antioxidant)	Interaction (P×T)	Source of variation	Storage period (P) (Days)	Treatment (T) (Antioxidant)	Interaction (P×T)
SEm	0.71	0.30	1.23	SEm	0.13	0.05	0.22
CD (0.05)	1.98	0.83	3.42	CD (0.05)	0.36	0.15	0.62
CV%	6.91			CV%	8.44		

In initial stage of storage peroxide value of ghee treated with 0.3% betel leaves was slightly higher compared to ghee added with BHA. However, upon further storage, the rise in peroxide values of BHA added ghee overtook peroxide value of betel leaves treated ghee. From 12th day of the storage peroxide value of BHA added ghee was significantly higher than of the betel leaves treated ghee and remained so during rest of the storage period.

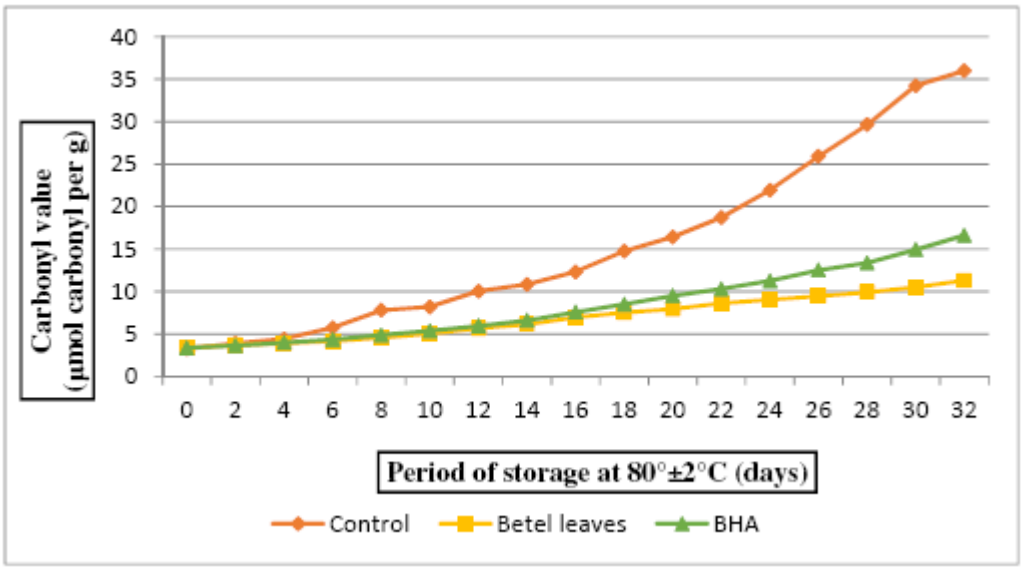

Figure 6: Changes in carbonyl value of ghee during storage at 80°±2°C after treating with different antioxidants

In starting of storage carbonyl value of the ghee treated with 0.3% betel leaves was almost at par with carbonyl value of the ghee added with BHA. However, upon further storage, the rise in carbonyl values of BHA added ghee overtook carbonyl value of betel leaves treated ghee. From 6th day of the storage carbonyl value of BHA added ghee was significantly higher than of the betel leaves treated ghee and remained so during rest of the storage period.

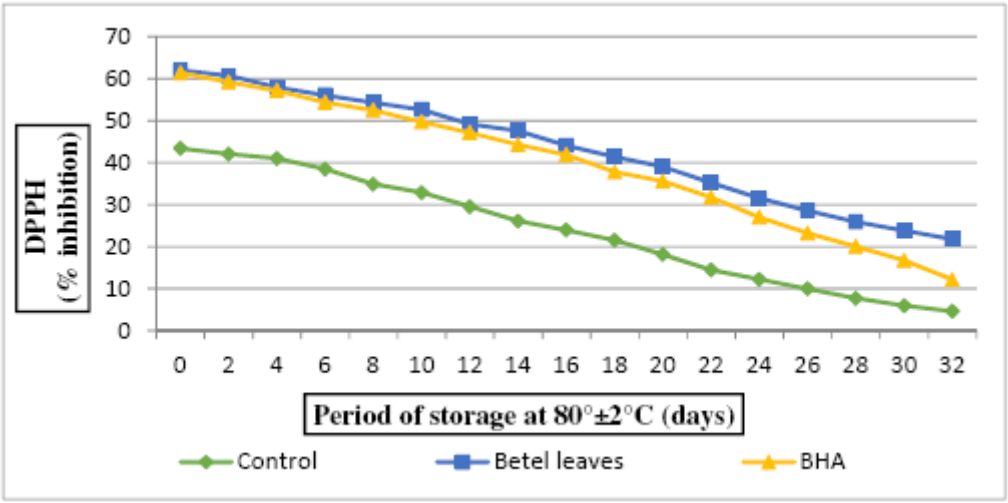

Figure 5(c): Changes in radical scavenging activity of ghee during storage at 80°±2°C after treating with different antioxidants

In starting of storage radical scavenging activity of the ghee treated with 0.3% betel leaves was almost at par with radical scavenging activity of the ghee added with BHA. From 2nd day of the storage radical scavenging activity of ghee treated with 0.3% betel leaves was significantly higher than that of the ghee added with BHA and remained so all throughout the storage period.

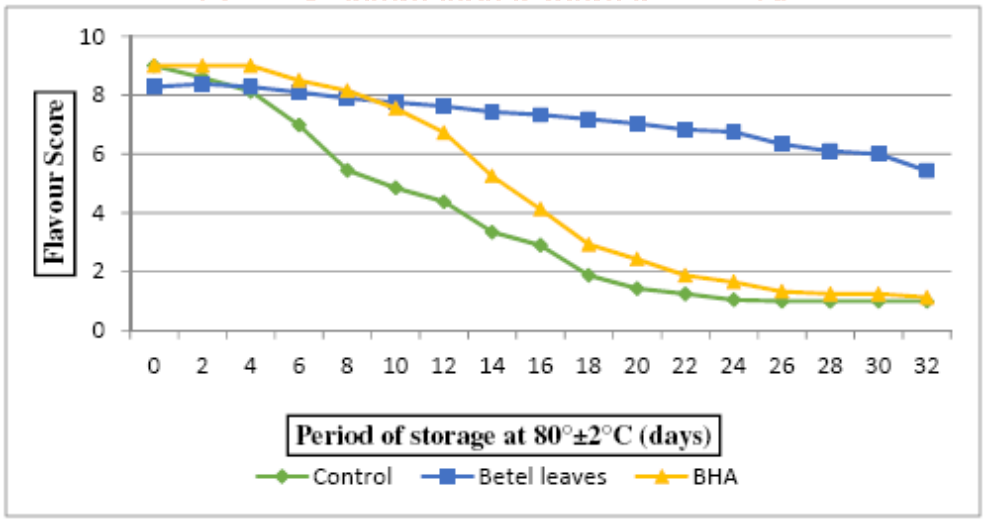

Figure 8: Changes in flavour score of ghee during storage at 80°±2°C after treating with different antioxidants

During initial stage of storage flavour score of ghee treated with 0.3% betel leaves was slightly lower compared to ghee added with BHA. However, upon further storage, the flavour score of BHA added ghee became lower than that of the betel leaves treated ghee. From 10th day of the storage flavour score of BHA added ghee was significantly lower than of the betel leaves treated ghee and remained so during rest of the storage period. The results of changes in flavour score on storage of the ghee samples were very well in corroboration with changes in peroxide value, carbonyl value and radical scavenging activity of the ghee samples.

Pradhan et al. (2013) studied polyphenols compounds in betel leaf extract and its ability to inhibit radiation induced lipid peroxidation process. They found that the presence of polyphenols compounds in betel leaf extract inhibited the radiation induced lipid peroxidation process effectively. This could be attributed to its ability to scavenge free radicals involved in initiation and propagation steps. The extracts reduced most of the Fe^{3+} ions and possess strong reductive ability. The extract also showed strong hydroxyl radical and superoxide anion radical scavenging property when compared with different standards such as ascorbic acid and BHT. Uddin et al.

(2015) and Nirmala and Kumari (2015) reported that the major terpenoids and phenols in betel leaves include 1,8-cineole, cadinene, camphene, caryophyllene, limonene, pinene, chavicol, allyl pyrocatechol, catechol, carvacrol, safrole, eugenol and chevibetel. Therefore, antioxidant properties of betel leaves in ghee as exhibited in present study might be attributed these compounds.

Patel and Rajorhia (1979) carried out the study dealing with the antioxidative effects of betel leaves when added to melted butter during clarification. The amount of betel leaves were 0.2, 0.5 and 1 per cent (w/v). A mixture of BHA and BHT (1:1) at concentration of 0.02 per cent by weight was also used. Ghee samples were stored at 30°C. The authors found that the initial flavour score of control sample and BHA+BHT added ghee sample was 8.33 and 8.3 respectively. Whereas, initial flavour score of 0.2, 0.5 and 1 per cent betel leaves added samples was 8.5, 8.2 and 8.4 respectively. After storage for 147 days the flavour score of control sample and BHA+BHT added ghee sample was 2.16 and 6.15 respectively. Whereas, after storage for 147 days flavour score of 0.2, 0.5 and 1 per cent betel leaves added samples was 2.2, 5.8 and 6.5 respectively. Therefore, results of present study are in line with the report. However, some deviation attributed to form of betel leaves used, stage of addition of betel leaves and temperature employed for storage of ghee. The authors used fresh betel leaves at initial stage of clarification and samples were stored at 30°C, whereas, in the present study betel leaves was used at final stage of clarification and samples were stored at 80°±2°C.

4. Conclusion

The study was conducted to evaluate potential of betel leaves (*Piper betel*) as an antioxidant in ghee to extend the shelf-life by retarding oxidative reactions during the storage of ghee. For addition of betel leaves in treatment of ghee final stage of heat clarification was found more effective than the initial stage of heat clarification. Treatment of ghee with different rate of betel leaves (0.1%, 0.2%, 0.3% and 0.4%), 0.3% rate was found more effective in reducing oxidative deterioration of ghee even more effective than BHA at 80°±2°C. Hence, betel leaves could be a very promising natural alternative over BHA.

Declarations

Author contribution statement

Kapadiya Dhartiben B: Conceived and designed the experiment and performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or Data; wrote the paper.

K.D. Aparnathi: Conceived and designed the experiments; Contributed materials, analysis tools.

Funding statement

This work was supported by Anand Agricultural University, Anand.

Competing interest statement

The authors declare that they have no competing interests.

Additional information

No additional information is available for this paper.

Acknowledgements

Anand Agricultural University, Anand for giving me fund to do this experiment.

References

[1] Basak, P., Mallick, P., Mazumder, S. and Verma, A. S. 2014. Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of tulsi (Ocimum sanctum) leaves. Int. J. Pharm. Res. Scholars. 3(1), 762-771.

[2] Benabdallah, A., Rahmoune, C., Boumendjel, M., Aissi, O. and Messaoud, C. 2016. Total phenolic content and antioxidant activity of six wild mentha species (Lamiaceae) from northeast of Algeria. Asian Pacific Journal of Tropical Biomedicine 6(9), 760-766.

[3] Bouayed, J., Bohn, T., 2010. Exogenous antioxidants—Double-edged swords in cellular redox state. Oxidative Medicine Cellular Longevity 3(4), 228-237.

[4] Brand-Williams, W., Cuvelier, M. and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und- Technologie, 28(1), 25-30.

[5] Chauhan, E. S., Aishwarya, J., Singh, A., Tiwari, A., 2016. A Review: Nutraceuticals properties of *piper betel*. American Journal of Phytomedicine and Clinical Therapeutics 4(2), 28-41.

[6] Chiang Chan, E. W., Wong, S. K., 2014. Phytochemistry and pharmacology of three *Piper* Species: An update. Int J Pharmacognosy 1(9), 534-544.

[7] De, S., 2004. Outlines of Dairy Technology, 19th edition, Oxford publishing Company, New Delhi.

[8] Duga, L., 1976. Lipids. In "Principles of Food Science. Part 1: Food Chemistry" (O. R Fennema, Ed.), Marcel Dekker, New York. PP. 169-186.

[9] Fukumoto, L. R., Mazza, G., 2000. Assessing antioxidant and pro-oxidant activities of phenolic compounds. Journal of Agricultural and Food Chemistry 48(8), 3597-3604.

[10] Gordon, M. H., 1990. The mechanism of antioxidant action in vitro. In Food Antioxidants (B. J. F. Hudson, Ed.), Elsevier Science Publishers Ltd Crown House, Linton Road, Barking, Essex IGll SJU, England. PP. 01-18.

[11] Hasim, Falah, S., Ayunda, R. D. and Faridah, D. N. 2015. Potential of lemongrass leaves extract (Cymbopogon citratus) as prevention for oil oxidation. Journal of Chemical and Pharmaceutical Research 7(10), 55-60.

[12] IS: SP: 18 (part XI). 1981. Handbook of Food Analysis: Dairy products. Bureau of Indian Standards, New Delhi. PP. 111.

[13] Jaiswal, S. G., Patel, M., Saxena, D. K. and Naik, S. N. 2014. Antioxidant properties of piper betel (L) leaf extracts from six different geographical domain of India. Journal of Bioresource Engineering and Technology 2(2), 12-20.

[14] Jamal, P., Barkat, A. A. and Amid, A. 2010. Distribution of Phenolics in Various Malaysian Medicinal Plants. Journal of Applied Sciences 10, 2658-2662.

[15] Jinesh, V. K., Jaishree, V., Badami, S. and Shyam, W. 2010. Comparative evaluation of antioxidant properties of edible and non-edible leaves of *Anethum graveolens* Linn. Indian Journal of Natural Product and Resources 1(2), 168-173.

[16] Kapadiya, D. B., Aparnathi, K. D., 2017. Evaluation of curry leaves (*Murraya koenigii*) for enhancing shelf-life of ghee against oxidative deterioration. Journal of Pharmacognosy and Phytochemistry 6(6), 951-962.

[17] Kapadiya, D. B., Aparnathi, K. D., 2017. Evaluation of liquorice (*Glycyrrhiza glabra*) for enhancing shelf-life of ghee against oxidative deterioration. *Int. J. Curr. Microbiol. App. Sci.* 6(12), 14555-1477.

[18] Kapadiya, D. B., Aparnathi, K. D., 2018. Evaluation of commonly used herbs to enhance shelf life of ghee against oxidative deterioration. Journal of Food Processing and Preservation 42(7), 01-13.

[19] Kapadiya, D. B., Dabhi, B. K., Aparnathi, K. D., 2016. Spices and herbs as a source of natural antioxidants for food. International Journal of Current Microbiology and Applied Sciences 5(7), 280-288.

[20] Kim, S., Jeong, S., Park, W., Nam, K. C., Ahn, D. U., Lee, S., 2006. Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. Food Chemistry 97, 472–479.

[21] Kumari, O. S., Rao, N. B., 2015. Phyto chemical analysis of *piper betel* leaf extract. World Journal of Pharmacy and Pharmaceutical Sciences 4(1), 699-703.

[22] Mehta, B. M., Aparnathi, K. D., Darji, V. B., 2015. Comparison of different methods of monitoring the secondary stage of oxidation of ghee. Int. J. Dairy Technol. 68(4), 589-594.

[23] Moure, A., Cruz, J. M., Franco, D., Domonguez, J. M., Sineiro, J., Domonguez, H., 2001. Natural antioxidants from residual sources. Food Chemistry 72(2), 145-171.

[24] Nicoliy, M. C., Anese, M., Parpine, M., 1999. Influence of processing on the antioxidant properties of fruit and vegetables. Tr. Food Sci. Technol. 10(3), 94-100.

[25] Nirmala, B. R., Kumari, O. S., 2015. Phyto chemical analysis of *Piper betel* leaf extract. World Journal of Pharmacy and Pharmaceutical Sciences 4(1), 699-703.

[26] Pakade, V., Cukrowska, E. and Chimuka, L. 2013. Comparison of antioxidant activity of Moringa oleifera and selected vegetables in South Africa. South African Journal of Science 109(4), 01-05.

[27] Patel, R. S., Rajorhia, G. S. 1979. Antioxidative role of curry (*Murrya koenigii*) and betel (*Piper betel*) leaves in ghee. Journal of Food Science and Technology 16(4), 158-160.

[28] Patel, S., Shende, S., Arora, S., Singh A. K., 2013. An assessment of the antioxidant potential of coriander extracts in ghee when stored at high temperature and during deep fat frying. International Journal of Dairy Technology 66(2), 207-213.

[29] Pawar, N., Arora, S., Bijoy, R., Wadhwa, B. K., 2012. The effects of *Asparagus racemosus* extract on oxidative stability of ghee, in relation to added natural and synthetic antioxidants. International Journal of Dairy Technology 65(2), 293-299.

[30] Pradhan, D., Suri, K. A., Pradhan, D. K., Biswasroy, P., 2013. Golden Heart of the Nature: *Piper betle* L. Journal of Pharmacognosy and Phytochemistry 1(6), 147-167.

[31] Rahman, M., Hossain, S., Rahaman, A., Fatima, N., Nahar, T. and Uddin B. 2013. Antioxidant activity of Centella asiatica (Linn.) Urban: Impact of extraction solvent polarity. Journal of Pharmacognosy and Phytochemistry 1(6), 27-32.

[32] Ronald, B. P., 2001. Spectrophotometric measurement of secondary lipid oxidation products. Current protocols in food analytical chemistry, John Wiley & Sons, Inc., New York.

[33] Shahidi, F., 2015. Handbook of antioxidants for food preservation. Woodhead Publishing Series in Food Science, Technology and Nutrition, UK. PP. 251-285.

[34] Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 16, 144-158.

[35] Song, F., Gan, R., Zhang, Y., Xiao, Q., Kuang, L. and Li H. 2010. Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. International Journal of Molecular Science 11(6), 2362-2372.

[36] Steel, R. G. D., Torrie, J. H., 1980. Principles and procedures of statistics- A Biometrical approach. 2nd Ed, McGraw- Hill, New York. PP. 137-167.

[37] Tupe, R. S., Kemse, N. G. and Khaire, A. A. 2013. Evaluation of antioxidant potentials and total phenolic contents of selected Indian herbs powder extracts. International Food Research Journal 20(3), 1053-1063.

[38] Uddin, F., Uddin, S. A., Hossain, D., Manchur, M. A., 2015. Antioxidant, cytotoxic and phytochemical properties of the ethanol extract of *Piper Betle* leaf. Int. J. Pharm. Sci. Res. 6(10), 4252-4258.