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Research Article

Effect of moringa pod powder on oxidative stability of ghee

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Abstract

Ghee, a fat-rich dairy product, undergoes oxidative deterioration under ambient conditions of storage. Use of natural antioxidants to retard such deterioration gained popularity. *Moringa oleifera* is considered a good source of natural antioxidants. The pod powder of *M. oleifera* was analyzed for its total phenolic content and radical scavenging activity. The total phenolic content and DPPH radical scavenging activity of moringa pod powder were found to be 587.16 mg GAE/100 g and 40.66 (% inhibition), respectively. Addition of moringa pod powder in melted butter during preparation of ghee was better in controlling oxidative deterioration of ghee. Changes in flavour score and peroxide value of ghee during accelerated storage suggested that 0.50 per cent pod powder was found best in retaining the flavour score of ghee by controlling oxidative deterioration. Effectiveness of the pod powder in enhancing oxidative stability of ghee with optimized rate (0.50 per cent) and stage of addition (in melted butter) was compared with BHA (0.02 per cent w/w) during storage at 80±2°C. The peroxide value and carbonyl value of ghee samples added with pod and BHA remained well below the control sample throughout the storage period. Therefore, addition of *Moringa oleifera* pod powder assisted to enhance the stability of ghee against oxidative deterioration.

Keywords: Ghee, Moringa pod powder, Oxidative stability, Peroxide value, Total phenolic content

Highlights

- Ghee is vulnerable to oxidative deterioration under ambient storage conditions.
- Moringa pod powder is considered a source of natural antioxidants.
- Moringa pod powder is better for controlling the oxidative deterioration of ghee when added to melted butter while making ghee.
- Moringa pod powder controls oxidative deterioration of ghee during accelerated storage as measured through changes in flavour score and peroxide value of ghee.
- The order of effectiveness in controlling oxidative deterioration in ghee samples was BHA > pod powder > control.

INTRODUCTION

Ghee, an anhydrous milk fat, is a traditional dairy product widely consumed in India. Oxidative rancidity is the major cause of ghee deterioration and is a limiting factor for shelf-life of ghee (Puravankara *et al.*, 2000; Mehta, 2006). Various approaches are used to improve the oxidative stability of fats. One of the most common approaches is addition of antioxidants. Antioxidants can be obtained naturally or synthetically prepared. As far as safety aspects are concerned, synthetic antioxidants manifested several adverse teratogenic, carcinogenic and mutagenic effects in experimental animals (Pawar *et al.*, 2012). It is known that natural antioxidants are efficient and safer than synthetic antioxidants. Natural antioxidant agents from plant sources have remarkably improved the shelf life of foodstuffs by retarding oxidative rancidity. The main sources of natural antioxidants are spices and herbs. *Moringa oleifera* is one of the potent sources of various compounds like flavonoids, phenolics and carotenoids, which can be used as antioxidants (Vongsak *et al.*, 2013). The phenolic compounds with antioxidant activity are gallic acid, p-cumaric acid, ferulic acid, caffeic acid, protocatechuic acid, cinnamic acid, catechin, epicatechin, vanillin and quercetin (Singh *et al.*, 2013). The antioxidant activity of the extract of leaves of *Moringa oleifera* was investigated and proved by Santos *et al.* (2012). Work reported for the antioxidant potential of *Moringa oleifera* in enhancing the shelf life of dairy products is scanty. This study evaluated the effect of moringa pod powder on oxidative stability of ghee under accelerated storage.

MATERIALS AND METHODS

Chemicals and glassware: Analytical grade (AR) chemicals were used in the study. Chemicals and

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glassware were supplied by authorized dealers.

Moringa pod powder: Moringa pod powder was purchased from the Pushpam Foods, Kunjrao (Anand), Gujarat.

Total phenolic content and radical scavenging activity of moringa pod powder: Total phenolic content of moringa pod powder was determined using Folin-ciocalteu's reagent according to the procedure described by Singleton and Rossi (1965). The radical-scavenging activity of moringa pod powder was determined as the ability to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals according to the procedure of Brand-Williams *et al*. (1995).

Preparation of ghee and addition of moringa pod powder: White butter for ghee preparation was procured from a commercial dairy plant. White butter was clarified (115°C for no hold) and allowed to cool until it attained a temperature of 80°C and filtered through six folds of muslin cloth in dry glass bottle.

Stage of addition of moringa pod powder during preparation of ghee: Moringa pod powder was added at two different stages during manufacture of ghee, i.e. (1) before heat clarification (in melted butter) and (2) at the final stage of heat clarification (i.e. when temperature reaches 115°C) @ 0.5 % of expected yield of ghee from butter. Ghee without addition of pod powder served as control. The ghee samples were subjected for accelerated storage (80±2°C). Changes in the peroxide value of ghee were monitored at intervals of three days. The study was carried out for up to 15 days. The stage for addition of pod powder giving better stability against oxidative deterioration of ghee was selected for further study.

Optimization of rate of addition of moringa pod powder in ghee: Moringa pod powder was added to the melted butter before heat clarification at the rate of 0.25, 0.50, 0.75 and 1.0 per cent (expected yield of ghee from butter). The ghee samples were stored at 80±2°C and analyzed for peroxide value and flavour score at 3 days of interval up to 15 days to determine optimized rate for further study.

Comparison of the antioxidant potential of moringa pod powder with synthetic antioxidant (BHA) added in ghee: To compare the antioxidative effectiveness of the pod powder with synthetic antioxidant butylated hydroxyl anisole (BHA) samples of ghee were prepared with pod powder at the optimized rate. Simultaneously, samples of ghee were prepared with BHA @ 0.02%. The sample of ghee without addition of pod powder served as a control. The samples of ghee obtained were stored at 80±2°C. The samples of ghee were monitored for oxidative changes by analyzing peroxide value, carbonyl value, and free fatty acid content at intervals of 3 days. Simultaneously, the samples were also monitored for changes in flavour score by sensory evaluation using 9-point hedonic scale. Moringa pod powder and BHA were compared for their antioxidant potential in ghee.

Determination of peroxide value: The peroxide value of ghee was determined by the iodometric method as given in IS: SP: 18 (part XI) BIS (1981).

Determination of carbonyl value: The carbonyl value of ghee was determined as per the method (Ronald, 2001).

Determination of free fatty acid content: The free fatty acid content of ghee was determined by the method given in IS: SP: 18 (part XI) BIS (1981).

Analysis of ghee for selected physico-chemical parameters: The fresh samples of ghee were analyzed for moisture content, Butyro-Refractometer reading at 40°C, Reichert Meissl value, Polenske value, free fatty acids, iodine value, saponification value and Baudouin Test by the methods as described in IS: SP: 18 (part XI) BIS (1981).

Statistical analysis: The data were analyzed using Analysis of Variance (ANOVA) at the 5% level of significance. Total five replications (n=5) were carried out. The data obtained for each of the attributes under study were subjected to statistical analysis in completely randomized design (Steel and Torrie, 1980).

RESULTS

Total phenolic content and radical scavenging activity of moringa pod powder

The total phenolic content (TPC) and radical scavenging activity of moringa pod powder was found to be 587.16±34.75 mg GAE per 100g and 40.66±2.24 (% inhibition), respectively.

Determination of the stage of addition of moringa pod powder in preparation of ghee

The changes in peroxide value of ghee samples (control and treated with pod powder) during accelerated storage (80±2°C) are presented in Fig.1.

The initial (i.e. on 0 day) peroxide values of the control ghee sample and the samples added with pod powder irrespective of stage of addition were zero. On further storage, peroxide values of the control ghee sample increased at a faster rate. On 15th day of storage, the peroxide value of the ghee sample added with pod powder in melted butter (7.94 meq O_2 per kg fat) was significantly lower than that of the ghee sample added with pod powder after heat clarification (9.11 meq O_2 per kg fat). The control ghee sample had a peroxide value of 13.02 meq O_2 per kg fat.

Optimization of rate of addition of moringa pod powder in ghee

The results obtained for changes in peroxide value and flavour score of ghee during accelerated storage (80±2°C) after treating with pod powder at different rates are depicted in Fig. 2 and Fig. 3. The moringa pod powder was added to the melted butter before heat clarification at the rate of 0.25, 0.50, 0.75 and 1.0 per cent (expected yield of ghee from butter). The statistical analysis of results revealed that the rate of pod powder addition and storage period have a significant effect ($P<0.05$) on changes in flavour score and peroxide value of ghee. The interaction effect of period of storage and treatment was also significant.

The initial (0 day) peroxide values of the control ghee and ghee added with pod at different rates were zero. During storage, peroxide value increased in all samples of ghee.

The flavour score of the control sample of fresh ghee was higher than that of the pod powder-added ghee samples. The flavour score of ghee decreased gradually up to 1.0 per cent addition of pod in the ghee. There was a significant difference ($P<0.05$) among treatments (different rates of addition). However, the score remained at an acceptable level up to 1.0 per cent addition of pod powder. On storage of ghee samples at 80±2°C, the flavour score of the control ghee sample decreased sharply and went below an acceptable level (<6) on the 9th day of storage. On the other hand, the flavour score of ghee samples added with 0.25, 0.50, 0.75 and 1.0 per cent pod went below acceptable levels on the 12th, 15th, 12th, and 12th day of storage, respectively.

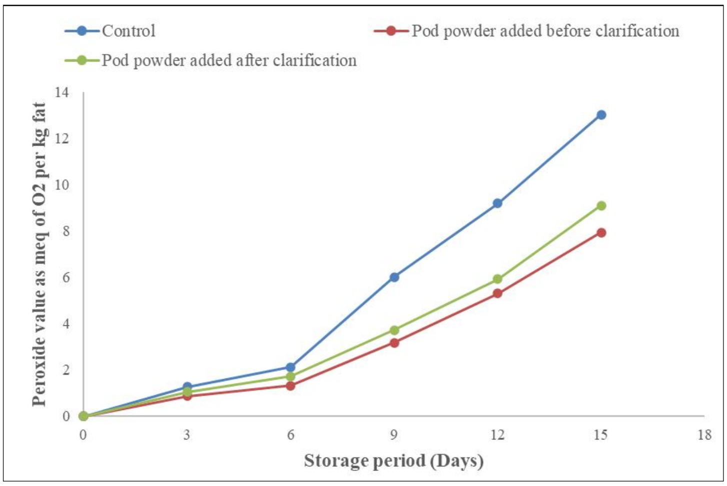

Fig. 1. Changes in peroxide value of pod powder treated ghee during accelerated storage

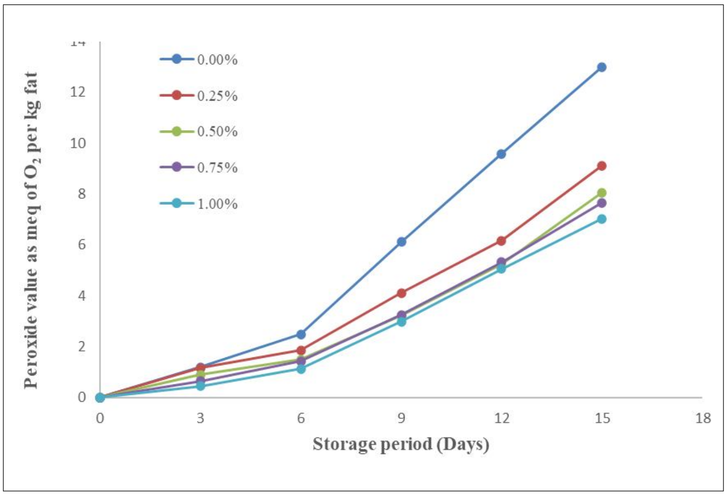

Fig. 2. Changes in peroxide value of ghee during accelerated storage after treating with pod powder at different rates

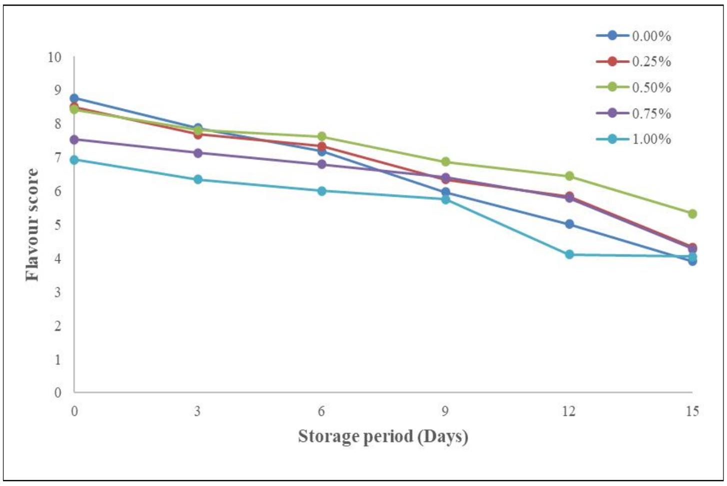

Fig. 3. Changes in flavour score of ghee during accelerated storage after treating with pod powder at different rates

Changes in flavour score and peroxide value of ghee during storage had conclusively suggested that addition of pod powder in ghee at the rate of 0.50 per cent was found best in retaining the flavour score of ghee by controlling oxidative deterioration. Therefore, among four different concentrations of pod used, the addition of pod at the rate of 0.50 per cent was selected for further study in this investigation.

Comparison of the antioxidant potential of moringa pod powder with synthetic antioxidant (BHA) added in ghee

The comparison was carried out using flavour score, peroxide value, carbonyl value, and free fatty acids content of the ghee samples.

Effect of moringa pod powder and BHA on flavour score of ghee: The results obtained for changes in flavour score of ghee are given in Table 1. On zero-day (when samples were fresh), flavour scores of control ghee and BHA-added ghee samples were almost similar. The flavour scores of the ghee sample containing pod powder were lower as compared to the control. During storage, the flavour score of the control sample declined at a faster rate. The flavour score of ghee samples added with pod powder was above the acceptable level on 12th day of storage. The order of acceptability for ghee samples was BHA > pod > control. On the 15th day of storage, only the ghee sample added with BHA had an acceptable flavour score.

Effect of moringa pod powder and BHA on peroxide value of ghee: The results obtained for changes in peroxide value of the ghee samples are given in Table 2. On zero day ghee incorporated with pod, BHA and control ghee samples showed zero peroxide value. On further storage, the peroxide value of the control ghee sample increased significantly. The peroxide value of ghee samples added with pod and BHA remained well below the control sample throughout the storage period. On the 15th day of storage, ghee samples incorporated with BHA, pod powder, and control had peroxide values of 14.25 meq O_2 per kg fat, 8.13 meq O_2 per kg fat and 6.10 meq O_2 per kg fat, respectively.

Effect of moringa pod powder and BHA on carbonyl value of ghee: The results obtained for changes in carbonyl value of the ghee samples are given in Table 3. On zero day ghee incorporated with pod, BHA and control ghee sample showed almost similar carbonyl value. On further storage, the carbonyl value of the control sample increased significantly. The carbonyl

Table 1. Changes in flavour score of ghee during accelerated storage (80±2°C)

Storage period (days)	Flavour score (out of 9)			
	Control	0.5% Pod powder	0.02 % BHA	Mean (Days)
0	8.52	8.37	8.47	8.45
3	8.11	7.82	8.18	8.04
6	7.01	7.53	7.85	7.46
9	6.17	6.80	7.30	6.75
12	5.15	6.26	6.77	6.06
15	3.77	5.25	6.12	5.05
Mean (Treatment)	6.46	7.00	7.45	

Source of variation	Storage period (P) (days)	Treatment (T)	Interaction (P×T)
CD (0.05)	0.11	0.08	0.19

Table 2. Changes in peroxide value of ghee during accelerated storage (80±2°C)

Storage period (days)	Peroxide value (meq of O_2 per kg fat)			
	Control	0.5% Pod powder	0.02% BHA	Mean (Days)
3	1.23	0.84	0.72	0.93
6	2.05	1.30	1.11	1.49
9	5.35	3.01	1.91	3.42
12	9.07	5.15	3.31	5.85
15	14.25	8.13	6.10	9.49
Mean (Treatment)	5.32	3.07	2.19	

Source of variation	Storage period (P) (days)	Treatment (T)	Interaction (P×T)
CD (0.05)	0.34	0.24	0.59

Note: In fresh ghee samples (0 day) peroxide value was found to be zero

Table 3. Changes in carbonyl value of ghee during accelerated storage (80±2°C)

Storage period (days)	Carbonyl value of ghee (µmol carbonyl per g)			
	Control	0.5% Pod powder	0.02 % BHA	Mean (Days)
0	4.72	4.93	4.65	4.77
3	5.98	6.18	5.90	6.02
6	6.81	6.38	6.46	6.55
9	8.62	7.58	7.02	7.74
12	10.90	9.40	7.93	9.41
15	14.36	11.72	8.87	11.65
Mean (Treatment)	8.57	7.70	6.81	

Source of variation	Storage period (P) (days)	Treatment (T)	Interaction (P×T)
CD (0.05)	0.59	0.42	1.02

Table 4. Changes in free fatty acid content of ghee during accelerated storage (80±2°C)

Storage period (days)	Free fatty acid content (% oleic acid)			
	Control	0.5% Pod powder	0.02 %BHA	Mean (Days)
0	0.158	0.157	0.151	0.155
3	0.190	0.169	0.160	0.173
6	0.275	0.226	0.200	0.234
9	0.346	0.293	0.253	0.297
12	0.436	0.380	0.294	0.370
15	0.549	0.452	0.371	0.457
Mean (Treatment)	0.326	0.280	0.238	

Source of variation	Storage period (P) (days)	Treatment (T)	Interaction (P×T)
CD (0.05)	0.02	0.01	0.03

value of ghee samples added with pod and BHA remained well below the control sample throughout the storage period. After completion of 15 days of storage, the lowest carbonyl value was observed in BHA added ghee sample (8.87 µM/g), followed by pod (11.72 µM/g). The control ghee sample had the highest carbonyl value (14.36 µM/g).

Effect of moringa pod powder and BHA on free fatty acid content of ghee: The results obtained for changes in free fatty acids of the ghee samples are given in Table 4. On zero day ghee incorporated with BHA showed lower percentage of free fatty acid content than pod and control ghee. After completion of 15 days of storage, the lowest free fatty acid content was observed in BHA added ghee sample (0.371%) followed by pod (0.452%). The control ghee sample had the highest free fatty acid content (0.549%).

The statistical analysis of results revealed that treatments and storage period have significant ($P<0.05$) effects on changes in flavour score, peroxide value, carbonyl value and free fatty acids of ghee during storage. The interaction effect of period of storage and treatment was also significant.

Table 5. Analysis of ghee for selected physico-chemical parameters

	Treatments		
Parameters	**Control**	**0.5% Pod powder**	**0.02% BHA**
Moisture (%)	0.227	0.239	0.231
BR reading at 40°C	42.13	42.20	42.35
RM value	29.50	29.89	29.73
Polenske value	1.36	1.40	1.32
FFA (% oleic acid)	0.158	0.159	0.150
Iodine value	32.12	32.23	32.17
Saponification value	225.06	223.86	224.49
Baudouin test	Negative	Negative	Negative

Values are mean of five replications

Analysis of ghee for selected physico-chemical parameters

The results obtained for selected physico-chemical parameters of ghee samples are presented in the Table 5. The moisture content in different ghee samples varied on an average from 0.227 to 0.239 per cent. BR reading of different ghee samples at 40°C varied on an average from 42.13 to 42.35. The RM value of different ghee samples varied on an average from 29.50 to 29.89. The Polenske value of different ghee samples varied on an average from 1.32 to 1.40. The FFA of ghee samples varied on an average from 0.150 to 0.159 per cent oleic acid. Baudouin test was found negative for all ghee samples.

DISCUSSION

Total phenolic content and radical scavenging activity of moringa pod powder: The total phenolic content and radical scavenging activity of moringa pod powder obtained in this study corroborated with the reported values in literature (Paliwal *et al*., 2011; Wangcharoen and Gomolmanee, 2011).

Stage of addition of moringa pod powder during the preparation of ghee: It is evident that treating the ghee with pod powder in melted butter reduced the peroxide formation more effectively compared to the ghee treated with pod powder at the final stage of clarification. This might be attributed to liberation of phenolic compounds from pod powder during heating of the butter. The contact time of pod powder with butter during heating was relatively more and might have facilitated release of more phenolic/flavonoid compounds from it. Therefore, the addition of pod before clarification, i.e. in melted butter, was carried out for further study. Antioxidative activity of a given compound may increase, decrease or remain unchanged as a function of temperature. The stability of an antioxidant in heat is advantageous in the food industry since many fat- and oil-containing foods are heated during processing (Brewer, 2011).

Optimization of rate of addition of moringa pod powder in ghee: The results indicated that addition of pod powder exerts resistance towards oxidative deterioration of ghee, and the effectiveness is observed to increase with the increase in the concentration of added pod powder. However, it is also observed that the effectiveness is not directly proportional to the quantity of added pod powder.

The flavour score of the control sample of fresh ghee was higher than that of the pod powder-added ghee samples. The flavour score of ghee decreased gradually up to 1.0 per cent addition of pod in the ghee. There was a significant difference ($P<0.05$) among treatments (different rates of addition). However, the score remained at an acceptable level up to 1.0 per cent addition of pod powder. The results indicated that the addition of pod powder in ghee at the rate of 0.50 per cent was most effective in retaining flavour score of ghee during storage.

Comparison of the antioxidant potential of moringa pod powder with synthetic antioxidant (BHA) added in ghee: In the present study, the order of exerting resistance towards oxidative deterioration in ghee samples was BHA > moringa pod powder > control.

Shende *et al.* (2014) studied the oxidative stability of ghee incorporated with clove extracts and BHA at elevated temperatures. Synthetic antioxidant, BHA showed higher antioxidant activity under accelerated storage conditions, whereas it had lower antioxidant potential during deep frying studies as compared to clove extracts (steam distilled and oleoresin).

Pawar *et al.* (2012) compared the potential of *Asparagus racemosus* (Shatavari) extract with natural (rosemary and green tea) and 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 natural and synthetic antioxidants.

Patel *et al.* (2013) observed that the coriander extracts (steam distilled and oleoresin) significantly inhibited ($P<0.05$) the peroxide development throughout the 21 days of storage at 80ºC as compared to the control. However, ghee incorporated with steam-distilled coriander extract showed a significant rise ($P<0.05$) in peroxide value as compared with its oleoresin counterpart. This indicated that ghee containing oleoresin is more effective in retarding peroxides development than ghee containing steam distilled extract. The peroxide value of ghee containing coriander extracts was significantly ($P<0.05$) higher than BHA throughout 21 days of storage at 80±1ºC.

Siwach *et al.* (2018) reported that incorporation of lycopene in preparation of buffalo ghee was found to be less effective in controlling oxidative rancidity in terms of free fatty acids content, peroxide value and thiobarbituric acid value compared with BHA at a level of 200 ppm.

Asha *et al.* (2015) assessed antioxidant activities of butylated hydroxyl anisole (BHA) and orange peel powder extract in ghee stored at elevated temperatures during the storage period of 21 days. Ghee incorporated with orange peel extract was found to have better resistance against the development of peroxides, thiobarbituric acid (TBA) and FFA than the ghee incorporated with BHA.

Addition of moringa pod powder exerts resistance towards the oxidative deterioration of ghee. Ghee incorporated with moringa pod powder, and BHA showed significantly lower peroxides development throughout 15 days of storage at 80±2ºC as compared with control ghee.

The carbonyl value of ghee samples added with moringa pod powder and BHA remained well below the control sample throughout the storage period. The obtained data were related to those of primary products of lipid oxidation determined as peroxide value. The incorporation of pod powder in ghee controlled the autoxidation of unsaturated double bonds and the formation of peroxides, hydroperoxides, and their decomposed products, principally carbonyl compounds. Addition of pod powder delayed the degradation of hydroperoxides to carbonyl compounds. Ghee incorporated with moringa pod powder, and BHA showed a significantly lower formation of carbonyl compounds during 15 days of storage at 80±2ºC as compared with control ghee.

Addition of moringa pod powder provided better storage stability. Ghee incorporated with pod powder, and BHA showed significantly lower free fatty acid content throughout 15 days of storage at 80±2ºC as compared with control ghee.

Asha *et al.* (2015) evaluated antioxidant activity of orange peel extract in ghee. The result revealed that a significant ($P<0.01$) increase in FFA was observed in all the ghee samples, while the least increase in FFA in ghee incorporated with orange peel extract and BHA throughout the storage period.

Shende *et al.* (2014) studied the oxidative stability of ghee incorporated with clove extracts and BHA at elevated temperatures. It was inferred that ghee samples containing BHA showed higher resistance against the development of FFA compared to those containing clove extracts (steam distilled and oleoresin). However, ghee incorporated with steam-distilled clove extract displayed lower FFA values than ghee containing clove oleoresin.

This study entails to conclude that addition of moringa pod powder helps to enhance the stability of ghee against oxidative deterioration. The changes in peroxide value of ghee during accelerated storage suggested that addition of moringa pod powder in melted butter was better in controlling oxidative deterioration of ghee to that of its addition at final stage of heat clarification. Changes in flavour score and peroxide value of ghee during accelerated storage suggested that 0.50 per cent pod powder was found best in retaining the flavour score of ghee by controlling oxidative deterioration. The order of effectiveness in controlling oxidative deterioration in ghee samples was BHA > pod powder > control.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contributions: MKC: Involved in investigation, data generation, and preparing original draft; AKJ: Engaged in conceptualization, data curation, and supervision; SCP, AIS: Engaged in analytical help and editing the manuscript.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

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