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INFLUENCE OF *PIPER BETEL* LEAVES ON THE SENSORY QUALITY AND PHYSICOCHEMICAL PROPERTIES DURING STORAGE STUDY OF GHEE

K.B. Kubade[1]*, B.D. Patil[2], D.D. Patange[3], D.K. Kamble[2] and C.V. Memane[3]

[1]P.G.I. M.P.K.V., Rahuri, Ahmednagar – 413 722, Maharashtra, India

[2]Department of Animal Husbandry and Dairy Science, P.G.I. M.P.K.V., Rahuri, Maharashtra, India

[3]Department of Animal Husbandry and Dairy Science, RCSM College of Agriculture, Kolhapur (M.H.), India.

*Corresponding author E-mail : kartikkubade@gmail.com

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ABSTRACT

This study was aimed to determine shelf life of *Piper betel* leaves added ghee. Three ghee samples were prepared where one was control, another one was BHA (Butylated Hydroxy Anisole) treated and the rest one was optimizing PBL(*Piper betel* leaves) in ghee at 1.5% of butter and the ghee samples were packed and sealed in glass jar and stored at 50! for storage studies. The samples were evaluated for sensory and physicochemical quality at 15days interval till the samples were sensorially unacceptable. In sensory evaluation flavour, texture, colour and freedom from suspended impurities (ghee residue) judged by '100' point scale and it was decrease significantly during storage. Samples were analyzed for moisture, peroxide value (PV), thiobarbituric acid (TBA), free fatty acids (FFA) value and conjugated diene (CD). Though storage period were increased PBL treated ghee samples PV, TBA, FFA, moisture and CD values were lowered significantly comparedto control samples. Moreover, 1.5% PBL added ghee samples values were lowered than BHA treated ghee sample. Therefore, it is concluded that PBL has great role for increasing shelf life of ghee.

Key words : Ghee, *Piper betel* leaves, Sensory qualities, Physico-chemical properties, Storage study.

Introduction

Ghee known as clarified butter or anhydrous milk fat (AMF). It is widely used in India since time immemorial. It is a fat-rich concentrated dairy product originally produced in India. It is prepared by clarifying butter fat to produce a characteristic flavor. Ghee has pleasing flavor and aroma; ghee has always a supreme status as an indigenous product in India (Ganguli and Jain, 1972) The major use of ghee is in frying and dressing foods, and it is considered a sacred article in some religious rites (Rajorhia, 1993). It is a highly nutritious dairy product with several therapeutic benefits (Deosarkar *et al*., 2016). Demand for ghee consumption is ever-increasing in India, where 35% of raw milk is transformed into ghee (Manjunatha, 2019).

According to FSSAI (2011) ghee means the pure clarified fats derived solely from milk to curd or from *desi* (cooking) butter or from cream to which no coloring matter or preservative has been added. On an average ghee contain fat (99.0-99.5%), moisture(<0.5%), carotene (3.2 to 7.4 mg/gm), vitamin A (19-34 IU/gm), cholesterol (209-312 mg/100 gm), tocopherol (18-31mg/g) and free fatty acid (2.8%) (Aneja *et al*., 2002), at least 98.8% of the triglycerides in ghee are made up of milk fat, and no more than 0.1% is water (Patange *et al*., 2015). Ghee is specifiedas containing a minimum 99.6% milk fat and 0.4% free fatty acid with no more than 0.1% moisture, Peroxide Value (PV) less than 1.0 (Codex Alimentarius, 2006; Sserunjogi, 1998). One gram of ghee supplies 9-kcal of energy (Walstra *et al*., 2006). Ghee is considered to be fairly stable due to the low water content and high anti-oxidative properties (Kwak *et al*., 2013).

Lipid is the major constituents and oxidation process in this play a key role in quality of ghee. Fat oxidation mainly depends on the several steps such as selection, storage, refining and manufacturing (Bhavaniramya *et al*., 2018) and oxidative degradation which includes hydrolysis, oxidation and polymerization followed by the formation of secondary products. Oxidation degrade ghee's color, flavor, aroma, nutritive value, shelf life and consumer acceptance, it also creates a negative effect on health status. The extent of degradation primarily depends on storage temperature, oxygen availability in packed or unpacked and ghee physical condition i.e., solid orliquid state of ghee (Pawar *et al*., 2014; Wasnik *et al*., 2017). Oxidative deterioration of ghee reduces not only its monetary value but also its appealing flavor, rendering it unappetizing and producing possibly hazardous chemicals (Duhan *et al*., 2020).

Ghee contains natural antioxidants like tocopherol, carotene, phospholipids (Kumar *et al*, 2018), but their concentration is too low to inhibit or delay oxidative deterioration. Synthetic antioxidants (*e.g.*, TBHQ, BHA and BHT are widely used as food additives, but their application has been re-assessed because of possible toxic or carcinogenic components formed (Yassari and Yasari, 2013). BHA is a permitted antioxidant in ghee @ 0.02% as per FSSAI (2011). Synthetic antioxidants have potential health risks to humans and the environment (Stich, 1991). It's found that antioxidants from natural sources like green tea, tulsi (Merai *et al*. 2003); rosemary, shatavari (Pawar *et al*., 2014) clove extracts (Shende *et al*., 2014), orange peel extract (Ahmed *et al*., 2022) and garden cress oil (Taha *et al*., 2022) reduce the oxidative stability in ghee and other oil and fat-rich products.

Recent years, there has been a growing interest in enhancing the nutritional value of milk and milk products (Kubade *et al*., 2023). PBL, also called as 'Paan' or 'Nagvalli' (family-*Piperaceae*), is an evergreen and perennial creeper. Everything from social interactions to religious beliefs to modern-day relevance has been discussed in relation to PBL and the aroma of PBL is due to the presence of essential oils, consisting of phenols and terpenes. (Sripadha, 2014). Betel leaf extract has a promising anti-carcinogenic role to play in tobacco induced cancer. (Padma *et al*., 1989). The presence of phenol and phenolic (Chavicol, Chavibetol, Chavibetol acetate and eugenol) in the PBL may be credulous to be responsible for its antioxidant activity (Kubade *et al*., 2021). The particular properties of PBL are antimicrobial properties (Sarkar *et al*., 2008). PBL also contain significant number of antioxidants like hydroxychavicol, eugenol, ascorbic acid and β carotene belonging to the propenyl phenol group (Chakraborty and Shah, 2011). PBL added ghee was sensorily superior with higher flavor and colour scores overall other treatments studied (Baburao *et al*., 2020). Therefore, a number of herbal food items having antioxidants properties have been used in food during processing to extend the shelf life and oxidative stability of products during storage (Dhiman *et al*., 2022).

Recently, PBL was fruitfully utilized in dahi (Sivakumar and Dhanalakshmi, 2015), papad (Vernekar and Vijayalaxmi, 2018), ice cream (Bhoite *et al*., 2019), flavored milk (Kamble *et al*., 2019) and sev (Jandu *et al*., 2022) and also utilized in ghee-based low-fat spread (Patange *et al*., 2023).

Looking to the interest of consumer towards food products preserved with natural antioxidants substances over chemical antioxidants it was plan to study the shelf life of ghee using PBL considering the nutritional, therapeutic and antioxidant properties of PBL it was planned to use in the preparation of ghee.

Materials and Methods

Preliminary trials were conducted to finalize level of PBL

The fresh and cleaned finely cut PBL at different levels (on weight basis of butter) was added in melted butter at 70-80°C during heating. The heating was continued till the temperature reaches up to 115°C. Ghee samples were prepared using different level of PBL. The samples of ghee were subjected to sensory evaluation and on the basis of these results; best level of PBL was selected for experimental trials.

Preparation of ghee

Ghee was prepared by creamery butter method as described by Pal (2002) with slight modification. Cross bred cow milk preheated to 35-40°C and then pass-through cream separator machine to get cream was inoculated with starter culture and kept for overnight period at room temperature and then butter was heated up to 70-80^{0}C and at that time addition of fresh PBL at selected level and thus optimized PBL in butter for preparation of ghee.

Chemicals and reagent : All the chemicals used were Analytical M/S Reagent (AR) and Guaranteed Reagent (GR) grade for analytical work which was manufactured by Merk, India Ltd/ Glaxo India Ltd.

Equipments : Cream Separator- Make Madhur Engineering Lt. Kolhapur, Spectrophotometer-Spectronic 20 Colorimeter (Geaesys 545TM Spectrophotometer, New York, USA).

Herb (PBL) : Fresh PBL of Calcutta variety were procured from local market of Kolhapur city.

Synthetic antioxidant : The synthetic antioxidant, butylated hydroxyl anisole (BHA) was obtained from Qualigens Fine Chemicals Ltd, Mumbai.

Addition of antioxidant

BHA was added directly to the freshly prepared ghee at the rate of 0.02% (w/w), respectively. Ghee without any added herbs extract served as control.

Technique of preparation of *Piper betel* leaves added ghee

The ghee samples were prepared by using the procedure described by Pal (2002) with some minor modifications. Ghee samples were prepared using best three levels of PBL selected from preliminary trials. The ghee samples were evaluated for sensory attributes and physico-chemical parameters

Reception of milk
↓
Pre-heating (35-40°C)
↓
Filtration
↓
Separation (using cream separator machine)
↓
Cream
↓
Fermentation (at 24°C for 10 hr)
↓
Churning (Hand churner)
↓
Butter
↓
Washing
↓
Melting (80 -90°C)
↓
Addition of *Piper betel* leaves / BHA (100°C) (As per given treatment)
↓
Clarification (115°C)
↓
Slow cooling
↓
Packaging and Storage (50±1°C)

Flowchart of Ghee Preparation

Storage study of ghee

From the result of sensory evaluation of PBL added fresh ghee samples, sensorily superior level, *i.e.*, 1.5 per cent level was selected for storage studies. *Piper betel* leaves added ghee sample was compared with ghee with and without synthetic antioxidant. The ghee samples were packed and sealed in glass jar and stored at 50°C for storage studies. The samples were evaluated for sensory and chemical quality on at 15 days interval till the samples were sensorily unacceptable.

The treatment details for storage studies were as follows:

$\mathbf{T_1}$: Ghee without additives (Control)

$\mathbf{T_2}$: Ghee with 0.02 percent BHA

$\mathbf{T_3}$: Ghee with fresh *Piper betel* eaves

Sensory quality of ghee

The ghee samples were subjected to sensory evaluation on a 100-point scale (IS 7770 – 1975) for following attributes: Flavor (score 50), Colour and appearance (score 10), Body and texture (score 30) and freedom from suspended impurities (score10).

Analytical work

The product was analyzed for moisture by Lal *et al*. (2011), peroxide value (AOAC965.33) FFA (Lal *et al*., 2011), Thio barbituric Acid (TBA) value as described by King (1962) and Conjugated dienes (Gosewadeet, 2017)

Statistical analysis

Data generated during the course of investigation were analyzed using factorial randomized design technique according to Snedecor and Cochran (1994).

Results and Discussion

Changes in sensory qualities of ghee during storage flavour

The data pertaining the change of flavour score of PBL and other ghee samples during storage are depicted in Fig. 1. The flavour score of T_1 sample was ranged 48.09±0.05 (0 day) to40.73±0.05 on 90th days of storage, while T_2 sample ranged from 48.08±0.11(0 day) to 37.08±0.07 on 120 days of storage and T_3 sample ranged from 48.10±0.03 (0 day) to 41.63±0.02 on 105th days of storage. In T_2 sample score for flavor was decrease significantly.

Heat treatment during the ghee, this results into the generation of carbonyls, free fatty acids (FFAs) and lactones, which are responsible for the development of ghee flavor (Kumbhare *et al*., 2021). The fresh control sample and PBL sample received full score for flavour. Same results were reported for coriander leaves, curry leaves, dill leaves and dodi treated fresh ghee samples by Kapadiya and Aparnathi (2018). Battula *et al*. (2020) reported that milk fat imparts excellent flavor and superior mouth feel to milk products. Deeth (2006) also reported that flavour defect caused by lipolysis and the defect are described as rancid, bitter, buytric or wintery during storage. Only betel leaves and curry leaves were found

promising in retarding flavor deterioration of ghee reported by Kapadiya and Aparnathi (2018).

Colour

The result with respect to colour of the ghee as affected by storage are presented in the Fig. 1. The data indicated that storage period progressed the score for colour decreased significantly ($p<0.05$) in all samples. In T_3 sample during first 15 days colour score was increased and then significantly decreased. However, the rate of decrease in score was higher in T_1 (Control) sample than T_2 and T_3 sample. The range of colour score in T_1, T_2 and T_3 score 9.73±0.08 to 7.80±0.01, 9.88±0.07 to 7.75±0.05 and 9.90±0.08 to 8.18 ±0.02, respectively.

The color of cow ghee varies from deep yellow to straw yellow reported by Bharwade *et al.* (2017). The yellow color of ghee is attributed to its B-carotene content, it undergoes oxidative degradation during storage resulting in an alteration of major quality parameters such as color, aroma and nutritive value was studied by Pawar *et al.* (2014). Kapadiya and Aparnathi (2018) reported that the golden yellow color of ajwain leaves, coriander leaves, and fenugreek leaves treated fresh ghee sample changed to light golden yellow color after the storage.

Texture

From Fig. 2, it was revealed that texture score was significantly decreased during entire period of storage. The score was decreased from 29.83±0.08 to18.26±0.03, 29.75±0.07 to 20.25±0.09 and 29.63±0.08 to 21.22±0.07 in treatment T_1, T_2 and T_3, respectively. The maximum decrease in texture score was recorded in T_1. Texture score in ghee sample was non significantly decreased in first 15 days in T_1, T_2 and T_3 sample, thereafter score was decreased significantly in all ghee sample during storage.

Bhavaniramya *et al.* (2018) reported that the texture or granularity of the ghee was also quality parameter which is identified by the presence of uniform size grains with very little liquid fat was desirable characteristic of food quality. Asha *et al.* (2015) reported that texture scores changed in up to 6 to 21 days of storage of control ghee and preservative ghee, respectively.

Freedom from suspended impurities (ghee residue)

The score for freedom from suspended impurities (ghee residue) in all sample decreased non-significantly ($p<0.05$) for first 30 days the score was significantly decreased during entire storage. The rate of decrease in sample without PBL and PBL added ghee observed same trend. The result as affected by storage are presented in the Fig. 2.

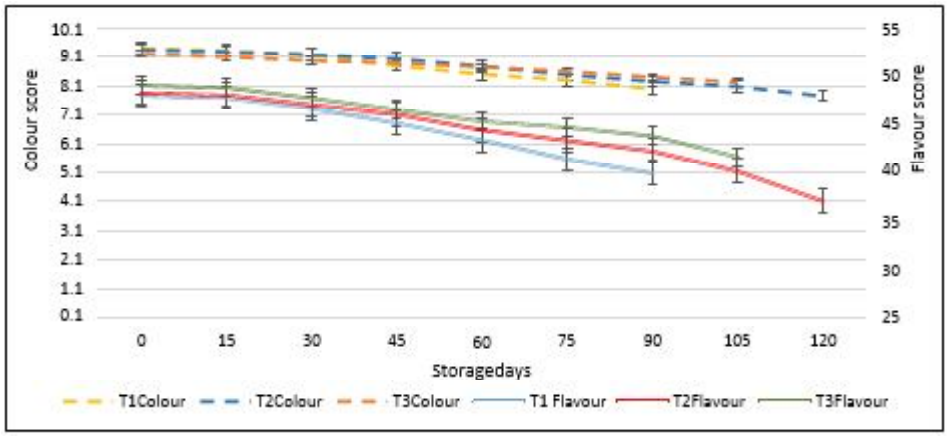

Fig. 1 : Changes in flavor and colour of ghee during storage.

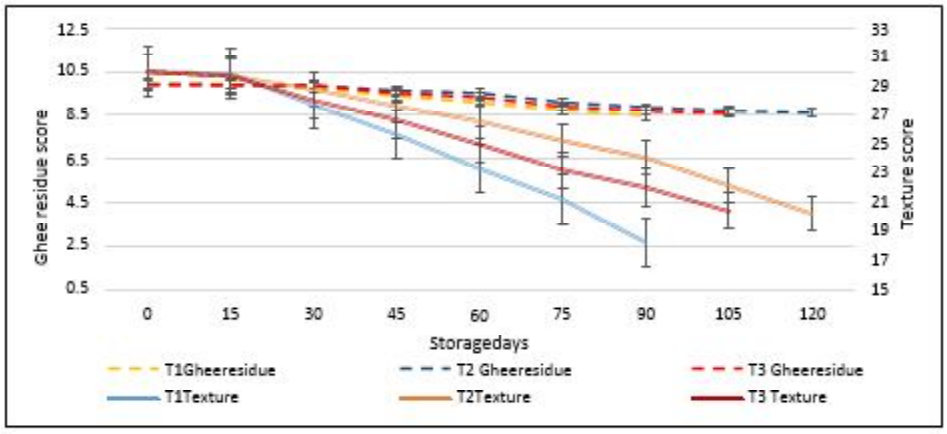

Fig. 2 : Changes in texture and ghee residue of ghee during storage.

Changes in physico-chemical properties of ghee during storage moisture

The changes in moisture content in stored ghee were depicted in the Fig 3. The data revealed that the moisture content of product was found increasing significantly as storage period progressed. In T_1 sample moisture content was increased from 0.23±0.001 to 0.53±0.006 per cent from zero day to 90th days of storage. Similarly, in T_2 sample moisture content 0.23±0.003 to 0.55±0.007 after 120 days of storage and T_3 sample 0.24±0.002 to 0.62±0.008 per cent after 105 days of storage. There was a significant ($p<0.05$) increase in moisture content of all three sample during storage.

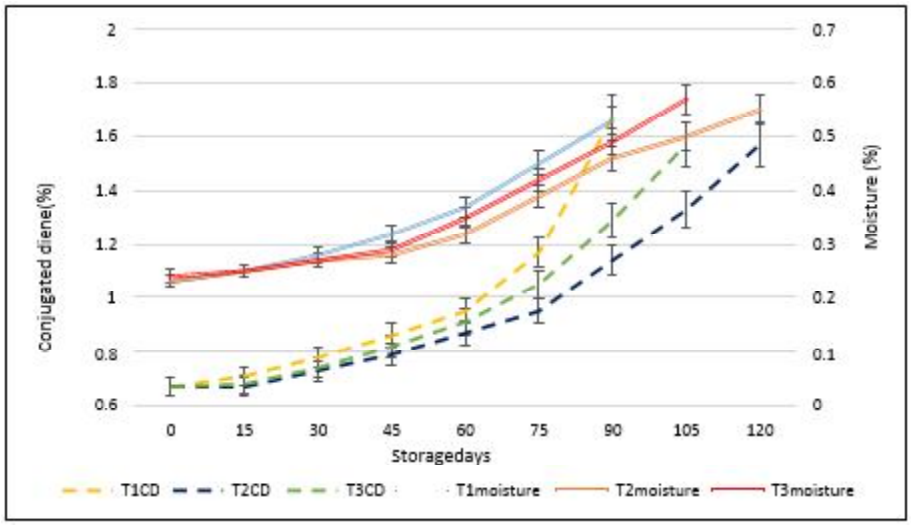

Fig. 3 : Changes in moisture and conjugated diene of ghee during storage.

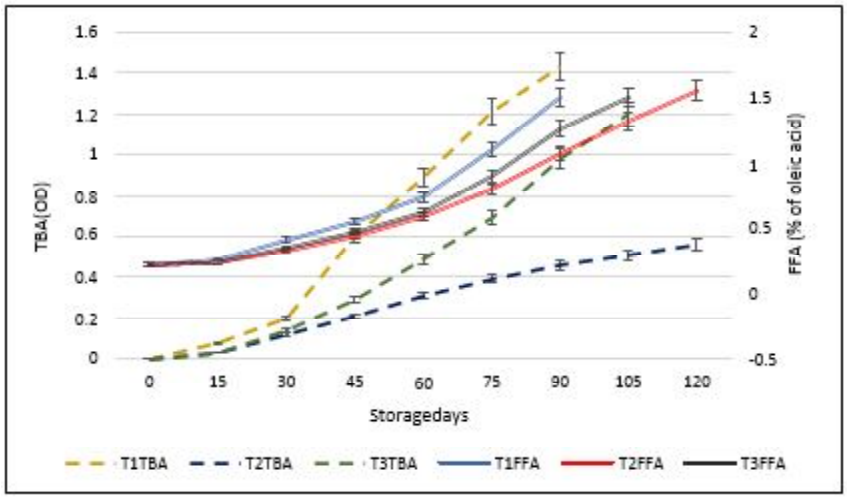

Fig. 4 : Changes in FFA and TBA of ghee during storage.

Similarly, Goyal *et al*. (2016) also mentioned the moisture percent in ghee ranged from 0.20 to 0.40 per cent. The moisture content of ghee was reported 0.3 per cent max. by Serunjogi *et al.* (1998). However, moisture content of cow milk ghee and buffalo milk ghee was reported less than 0.5 per cent by Illingworth *et al*. (2009).

Conjugated diene

The data presented in fig 3.0, revealed that changes in conjugated diene per cent during storage of PBL added ghee and other two samples of ghee. Conjugated diene per cent increased from 0.67±0.008 to1.67±0.006, 0.67±0.009 to1.57±0.011and from 0.67±0.004 to 1.57±0.011 in sample T_1, T_2 and T_3, respectively. In all sample first 30 days the score ~~a~~ decreased non-significantly ($p<0.05$). It means that there was a significant ($p<0.05$) increase in conjugated diene value in all three sample during storage. BHA (0.02%) delayed the development of conjugated dienes in comparison with the control ghee sample which showed progressive development of conjugated dienes throughout 21 day of storage at 80ºCwas studied by Hirabhai (2006).

Free fatty acid

The stored samples were analyzed for FFA content with a view to monitor the lipolytic changes during storage, which could affect the acceptability of the product. A gradual increase of FFA in all three sample of ghee with irrespective treatment was noticed during storage fig 4.0,The rate of increase was higher in the control (T_1), where FFA increased from initial 0.22±0.006 to 1.38±0.008 per cent (% oleic acid) after 90th days of storage. Then rate of increase in FFA content of BHA from initial 0.22±0.004 to 1.48±0.011 per cent (% oleic acid) after 120th days of storage and sample of PBL added ghee (T_3) from initial 0.22±0.005 to 1.42±0.004 per cent (% oleic acid) after 105th days of storage. There was a significant ($p<0.05$) increase in FFA in all three sample during storage.

Free fatty acids are undesirable in the milk fat products and responsible for the rancid flavour as reported Borgstrom and Jonson (1986)., Munro *et al*. (1992). Patel and Rajoria (1979) mentioned in ghee free fatty acid content increase when storage at 30ºC and observed that betel leaves at 1.0% concentration provided maximum protection against the hydrolysis of ghee.Aditya and Divya (2018) found that changes in FFA value of all treatment sample were 0.22 to 0.38, 0.22 to 0.49 and 0.22 to 0.44 per cent of oleic acid, respectively. The ghee with PBL also competes with ghee containing synthetic preservative may be due to antioxidant property of PBL as reported by Chakraborthy and Shah (2011), Sarma (2018). Similarly, Swapna *et al*. (2012) also mentioned the antioxidant property of PBL.

TBA value

The changes in TBA number of the control ghee, with PBL added and synthetic preservative ghee during storage are presented in Fig 4. In T_1 sample TBA value was increased from 0.00±0.006 to 1.43±0.008 from 0 day to 90th days of storage. Similarly, in T_2 and T_3 sample TBA value increased from 0.00±0.005 to 0.56±0.005 per cent on 120th days of storage and 0.00±0.004 to

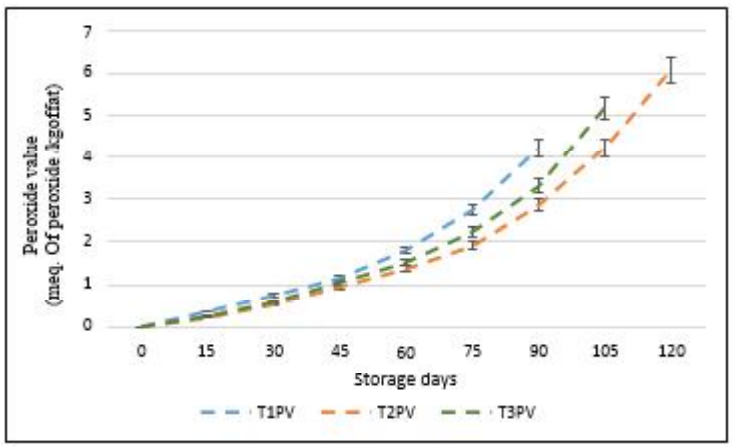

Fig. 5 : Changes in peroxide value of ghee during storage.

1.30±0.006 percent on 105th days of storage at 50°C. There was a significant ($p<0.05$) increase in TBA value in all three sample during storage.

The effect of storage period on change in TBA value of ghee 0.01 to 0.52 reported by Aditya and Divya (2018). Asha *et al*. (2015) reported that storage of ghee in accelerated temperature up to 21days observed initially no rise in TBA number but after accelerated storage days which became increased higher compared to orange peel extract added ghee. Patange *et al*. (2013) reported that TBA value remained unchanged during first 2week of storage.

Peroxide value

The stored samples were analyzed for peroxide value (PV) with a view to monitor the changes during storage, which could affect the acceptability of the product. A gradual increase of PV in all three sample of ghee with irrespective treatment was noticed during storage in Fig. 5. The rate of increase was higher in the control (T_1) wherein peroxide value increased from initial 0.00±0.002 to 4.21±0.011 on 90th days of storage and synthetic preservative (BHA) added ghee (T_2) from 0.00±0.004 to 6.08±0.012 on 120th days of storage. Then rate of increase in PBL added ghee (T_3) PV increased from initial 0.00±0.004 to 5.19±0.010 on 105th days of storage. There was a significant ($p<0.05$) increase in PV in all three sample during storage.

The development of peroxide value during the storage at 37°C in fresh control cow ghee and added BHA in ghee, there was a progressive increase the peroxide value in the entire sample after 8 months of storage (Gosewade *et al*., 2017). The change in PV (meq.kg) of margarine from 0.52 to 2.33 meq./kg during storage was also recorded by Zaeroomali *et al*. (2014). It was observed that the herb like *Piper betel* could successfully lowered the PV of ghee and this finding is a close agreement with reported by Pawar *et al*. (2014).

Conclusion

The rate of increase in Free Fatty Acid, Peroxide value, Thiobarbituric acid (TBA) value and Conjugated diene were significantly lowered in the experimental ghee over the ghee prepared without *Piper betel* leaves.

The rate of decreased in flavour, texture, colour and freedom from suspended impurities (ghee residue) were slight, but significantly decreased in the experimental ghee over the ghee prepared without *Piper betel* leaves.

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Abbreviations: BHA: Butylated Hydroxyl Anisole; **CD:** Conjugated Diene; **FFA:** Free Fatty Acid, **PBL:** *Piper betel* Leaves, **PV:** Peroxide Value, **TBA:** Thio Barbituric Acid.