INFLUENCE OF Piper betel leaves ON THE SENSORY QUALITY AND PHYSICOCHEMICAL PROPERTIES DURING STORAGE STUDY OF GHEE


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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 15 days 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 compared to 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.

ABSTRACT

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 (18-31 mg/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% of water (Patange et al., 2015). Ghee is specified as 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 (Bhavaniramy 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 oil/liquid 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°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.

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

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 (score 10).

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 T1 sample was ranged 48.09±0.05 (0 day) to 40.73±0.05 on 90th days of storage, while T2 sample ranged from 48.08±0.11 (0 day) to 37.08±0.07 on 120 days of storage and T3 sample ranged from 48.10±0.03 (0 day) to 41.63±0.02 on 105th days of storage. In T2 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, butyric or wintry 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 T₁ sample during first 15 days colour score was increased and then significantly decreased. However, the rate of decrease in score was higher in T₁ (Control) sample than T₂ and T₃ sample. The range of colour score in T₁, T₂ and T₃ 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 to 18.26±0.03, 29.75±0.07 to 20.25±0.09 and 29.63±0.08 to 21.22±0.07 in treatment T₁, T₂ and T₃, respectively. The maximum decrease in texture score was recorded in T₁. Texture score in ghee sample was non significantly decreased in first 15 days in T₁, T₂ and T₃ 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.

**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 T₁ 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 T₂ sample moisture content 0.23±0.003 to 0.55±0.007 after 120 days of storage and T₃ 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.
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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 to 1.67±0.006, 0.67±0.009 to 1.57±0.011 and from 0.67±0.004 to 1.57±0.011 in sample T₁, T₂ and T₃, 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 80°C was 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 (T₁), 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 (T₃) 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 30°C 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 T₁ 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 T₂ and T₃ 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...
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 21 days 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 2 weeks 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 (T₁) 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 (T₂) from 0.00±0.004 to 6.08±0.012 on 120th days of storage. Then rate of increase in PBL added ghee (T₃) 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).

1. 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.

References


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(Murrya koenigi) and betel (Piper betel) leaves in ghee. *J. Food Sci. Technol.*, **16**, 158.


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