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Process optimization for ghee added with betel vine (*Piper betel*) leaves

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Abstract
Ghee of its pleasing flavor and aroma, ghee has always had a supreme status as an indigenous product in India. ghee will boon to the Indian Dairy Industry, as in excess milk production most of the butter fat is stored in the form of ghee and it is a most logical approach. However, ghee is more prone to the oxidation and therefore deterioration may occur. To control oxidation synthetic anti-oxidant are generally used. At the same time synthetic anti-oxidant have several health hazards. Therefore, here an attempt was made to develop protocol for utilization of *Piper betel* leaves in ghee with increased shelf-life. The various levels of *Piper betel* was added in ghee *viz*., 1.0 (L_1), 1.5 (L_2) and 2.0 (L_3) per cent. the level was optimized on the basis of sensory evaluation and physico chemical properties. It was found that the ghee prepared by the addition of the *Piper betel* leaves 1.5 per cent obtained maximum scored for all the sensory qualities among other two level under study. In trial the results showed that the flavour, texture, colour and freedom from suspended impurities score of ghee was recorded maximum for ghee prepared by using *Piper betel* leaves of 1.5 (L_2) per cent level. Also, moisture, free fatty acid, peroxide value, TBA value, Conjugated diene was recorded maximum score for ghee prepared by using *Piper betel* leaves of 1.5 (L_2) per cent level. Consumers as a whole liked the product 'moderately' to 'very much' with an average score of 7.70.

Keywords: Ghee, *Piper betel* leaves, sensory evaluation, physico-chemical properties

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. It is a highly nutritious dairy product with several therapeutic benefits (Deosarkar *et al*., 2016) [7]. It has been an integral part of our food culture. Ancient Sanskrit literature describes, Ghee (Ghrita) as the food fit for gods and commodity of enormous value. Milk fat occurs naturally in milk and cream forming occurred oil in water emulsion properties (Ronholt *et al*., 2013) [20].
According to FSSAI (2011) [8] ghee means the pure clarified fats derived solely from milk to curd or from *desi* (cooking) butter or from cream to which no colouring 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), Vit 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) [2].
Ghee is almost anhydrous milk fat and there is no similar product in other countries. It is by far the most ubiquitous indigenous milk product and is prominent in the hierarchy of Indian dietary. Being a rich source of energy, fat soluble vitamins and essential fatty acids, and due to long shelf life at room temperature (20 to 40˚C), ghee is manufactured mostly from buffalo milk. due to lack of carotenoids in buffalo milk, ghee prepared from milk is white unlike cow ghee which has a golden yellow color. Because of its pleasing flavor and aroma, ghee has always had a supreme status as an indigenous product in India. (Ganguli and Jain, 1972) [9]
The monounsaturated and polyunsaturated fatty acids of ghee are susceptible to oxidative free radical reaction which is referred to as autooxidation and is a multi-step process. The rate of oxidation increases by itself as the reaction proceeds under usual processing and storage conditions. Such undesirable changes often create economic losses to dairy industry. The oxidative rancidity in ghee is one of the severe problems thereby affecting the sensory characteristics and loss of nutritional quality. Several workers have carried out exhaustive work to improve the stability of ghee against auto oxidation by altering processing parameters using proper packaging practices and storage conditions, addition of milk components and use of antioxidants.

The chemical composition of ghee revealed that lipid is the major constituents and this oxidation process 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) [5]

BHA is a permitted antioxidant in ghee @0.02% as per FSSAI, 2011 [8]. However, BHA has shown to cause lesion formation in rat fore stomach. Synthetic antioxidants (e.g., TBHQ, BHA and BHT) are widely used as food additives, but their application has been reassessed because of possible toxic or carcinogenic components formed during their degradation. Consequently, the search for endogenous protective ingredients in foods has been intensified wherein their utilization requires only manipulation of food formulations. A number of natural antioxidants have been added during food processing and have elongated the shelf life and oxidative stability of stored products.

The *Piper betel* leaf commonly known as 'paan' or 'Nagvalli' (family-Piperaceae) is an evergreen and perennial creeper significance of leaves has been explained in relationship to every sphere of human life including social, culture, religious and very much relevant even in modern days. From ancient time *Piper betel* are chewed along with areca nut, slaked lime, cardamom and clove in many Asian countries. Various properties of betel leaves include antioxidant, antifungal, antidiabetic, antimicrobial, anti-inflammatory, antifertility, antinacepative and radioprotective properties, (Sripradha, 2014) [27]. Betel leaf extract has a promising anticarcinogenic role to play in tobacco induced cancer. (Padma *et al.*, 1989)

The particular properties of *Piper betel* leaves are antimicrobial and antileshmian properties. *Piper betel* leaves have long been studied for their diverse pharmacological actions (Sarkar *et al*., 2008) [21] To date, the numerous studies carried out on the essential oil composition of *Piper betel* L. have identified five chemical group depending on the predominance of particular compounds *viz*. Alkaloid amide group, propenylphenol group, terpene sesquiterpene groups, steroid group, prenylated hydroxybenzoic acid group (Singtongratana, *et al.*, 2013) [24]. *Piper betel* leaves also contain significant amount of antioxidants like hydroxychavicol, eugenol, ascorbic acid and ß carotene belonging to the propenylphenol group (Chakraborty and Shah, 2011) [6]. The presence of phenol and phenolic (Chavicol, Chavibetol, Chavibetol acetate and eugenol) in the *Piper betel* leaves may be credulous to be responsible for its antioxidant activity (Swapna *et al.*, 2012) [29].

Considering the nutritional, therapeutic and antioxidant properties of *Piper betel* leaves it is planned to use the *Piper betel* leaves in the preparation of ghee.

Materials and Method

Fresh milk of crossbred cow was procured from Dairy Farm, RCSM College of Agriculture Kolhapur, which was separated to obtain cream. Fresh Piper betel leaves of local variety cultivar were procured from local market of Kolhapur city. BHA from Qualigens Fine Chemicals Ltd, was used as synthetic antioxidants. Cream Separator Make Madhur Engineering Lt. Kolhapur was used to separate cream from milk Spectrophotometer Spectronic 20 Colorimeter (Geaesys 545TM Spectrophotometer, New York, USA) was used for analysis purpose. All the glasswares of Borosil make were used for analysis of product. 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/ GlaxoIndia Ltd.

Methodology

Preparation of ghee

Ghee was prepared by creamery butter method as described by Pal (2002) [17] with slight modification. Crossbred cow milk was preheated (35 to 40°C) and then pass through cream separator machine to get cream, which was inoculated with starter culture and kept for overnight period at room temperature. On next day the fermented cream was churned to obtain butter. The obtained butter was used for preparation of ghee.

Preliminary trials

Preliminary trials were conducted to finalize level of *Piper betel* leaves.

The fresh and cleaned finely cut *Piper betel* leaves at different levels (on weight basis of butter) were added in melted butter at 100°C during heating. The heating was continued till the temperature reaches up to 115°C. Ghee samples were prepared using different level of *Piper betel* leaves i.e. PL_0, PL_1, PL_2, PL_3, PL_4, and PL_5.The samples of ghee were subjected to sensory evaluation and on the basis of these results, best three levels of *Piper betel* leaves were selected for experimental trials.

The treatment details for experimental trials for evaluation of fresh ghee samples are as below:

L1: *Piper betel* leaves @ 1.0% of butter
L2: *Piper betel* leaves @ 1.5% of butter
L3: *Piper betel* leaves @ 2.0% of butter

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

Physico-chemical analysis

The product was analysed for moisture of ghee given by (Lal *et al*. 2011) [16], peroxide value of ghee samples was determined by Lea's method as described in AOAC 965.33, Free Fatty Acid was determined by (Lal *et al*., 2011) [16], Thiobarbituric Acid (TBA) Value (King 1962) [15] and Conjugated dienes (Gosewade *et al*., 2017) [10].

Statistical Analysis

Data generated during the course of investigation were analyzed using completely randomized design (CRD) technique according to Snedecor and Cochran (1994) [25].

Experimental trials

The ghee samples were prepared by using the levels of *Piper betel* leaves finalized in preliminary trials. The fresh ghee samples prepared under different treatments were subjected to sensory evaluation and physico chemical analysis.

Effect of level of *Piper betel* leaves on sensory qualities (score) of fresh ghee

Flavour

From table 1 and fig. 1, it was observed that average flavour

score of *Piper betel* leaves added ghee for Level L_0, L_1, L_2 and L_3 were 47.80±0.004, 47.98±0.007, 48.23±0.006 and 46.82±0.008 respectively. The level L_2 was scored the highest flavor score (48.23±0.006) and the level L_3 had lowest flavour score (46.82). The higher level of *Piper betel* leaves incorporation resulted in slightly strong and astringent flavour of *Piper betel* leaves. flavour score decreased as the level of *Piper betel* leaves increased. The flavour score of ghee was significantly influenced by the levels *Piper betel* leaves. As *Piper betel* leaves level increased from 1.0 per cent 1.5 per cent flavor of the product increased, however *Piper betel* leaves level beyond 1.5 per cent, the flavor score of the product declined. It might be due to higher *Piper betel* leaves level might be masked original flavor of ghee and flavor scores were declined.

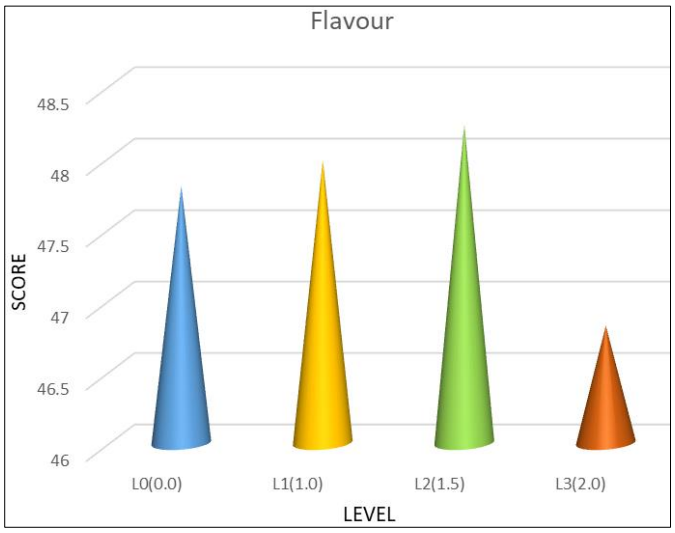

Fig 1: Effect of level of *Piper betel* leaves on flavor (score) of fresh ghee

Strong aromatic smell of *Piper betel* leaves was also reported by Satyal *et al*. (2012). Patel and Rajorhia (1979) [22, 18] noticed that when betel (*Piper betel*) and curry (*Murrayakoeniji*) leaves were added to butter during clarification in to ghee, the samples of ghee were rated excellent the judges preferred ghee samples treated with betel and curry leaves. Therefore, acceptability of the betel leaves and curry leaves treated ghee samples for flavor in sensory evaluation was very well. (Kapadiya 2018) [14]. Improvement in taste and aroma of papad by incorporation of *Piper betel* leaves was observed by Vernekar and Vijayalaxmi (2018) [30]. Whereas 7.5 to 8.5 score for flavoured milk added with *Piper betel* leaves extract was recorded by Kamble *et al.* (2019) [13].

Texture

From table 1 and fig. 2 it was revealed that the level of *Piper betel* leaves has non-significant effect on texture score of ghee samples. The score of texture of ghee was recorded higher in L_0 (29.82) sample of without *Piper betel* leaves ghee, however levels L_1, L_2 and L_3 are at par with each other. Aditya and Divya (2018) [1] reported that effect of different levels of alcoholic extract of *Piper betel* leaves on texture score was non-significant.

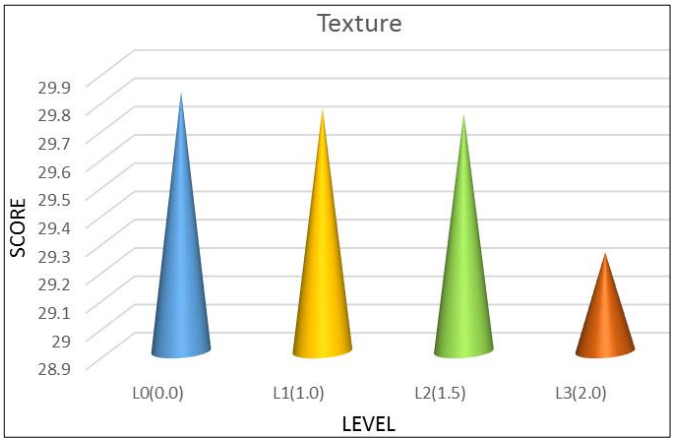

Fig 2: Effect of level of *Piper betel* leaves on texture (score) of fresh ghee

Table 1: Effect of level of *Piper betel* leaves on sensory qualities (score) of fresh ghee

Treatment	Sensory attribute			
	Flavor	Texture	Colour	Freedom from suspended impurities
L_0(0.0)	47.80^{b}±0.004	29.82^{bc}±0.04	8.40^{b}±0.05	9.98^{b}±0.005
L_1(1.0)	47.98^{c}±0.007	29.76^{b}±0.03	9.44^{c}±0.03	9.96^{b}±0.006
L_2(1.5)	48.23^{d}±0.006	29.74^{b}±0.01	9.94^{d}±0.04	9.95^{b}±0.004
L_3(2.0)	46.82^{a}±0.008	29.25^{a}±0.02	8.85^{a}±0.02	9.88^{a}±0.003
S.Em	0.008	0.01	0.05	0.007
CD(P< 0.05)	0.02	0.03	0.16	0.02

*mean ± SE (five)

Colour

From table 1 and fig. 3 it was revealed that mean sensory scores for colour for levels L_0, L_1, L_2 and L_3 were 8.30±0.05, 9.44±0.03, 9.94±0.04 and 8.12±0.02 respectively. The significant differences were observed in between these 4 treatments. This might be due to increased level of *Piper betel* leaves. The colour score of ghee was significantly influenced by the level of *Piper betel* leaves. It was observed that, as *Piper betel* leaves level increased, colour score was increased up to 1.5 per cent but above 1.5 per cent scores declined as it gives intense colour to product.

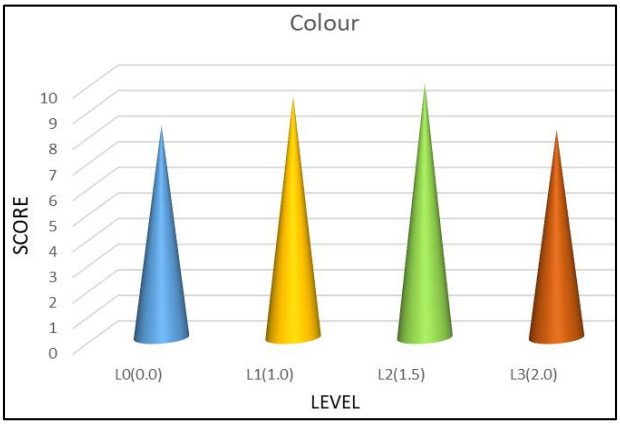

Fig 3: Effect of level of *Piper betel* leaves on colour (score) of fresh ghee

Patel and Rajorhia (1979) [18] noticed that when betel (*Piper betel*) and curry (*Murrayakoeniji*) leaves were added to butter during clarification in to ghee, the samples of ghee treated with betel leaves and curry leaves were appreciated for slightly higher intensity of their color. The effect of level of *Piper betel* leaves on colour and appearance score of flavoured milk was also studied by Kamble *et al.* (2019) [13] who found that the colour and appearance score of *Piper betel* flavoured milk ranged from 7.5 to 8.6. The maximum score was obtained for formulation, which had 5 per cent *Piper betel* leaves extract and 10 per cent sugar.

Freedom from suspended impurities

From table 1 and fig. 4 it was revealed that the maximum score of suspended impurities of ghee was recorded by the 0 per cent *Piper betel* leaves (L_0) 9.98±0.005. It might due to absence of small suspended particle of *Piper betel* leaves in ghee. In case of increase the level of *Piper betel* leaves in ghee decrease suspended impurities score. Scores for levels L_1, L_2 and L_3 were 9.96±0.006, 9.95±0.004 and 9.88±0.003, respectively. The levels L0, L1 and L2 are at par with each other. The reason behind this might be slight increase in the *Piper betel* leaves suspended particle content in ghee with increase the level of *Piper betel* leaves.

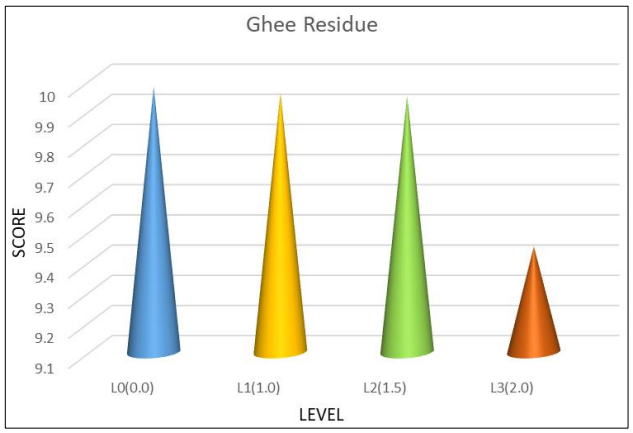

Fig 4: Effect of level of *Piper betel* leaves on freedom from suspended impurities (ghee residue) score of fresh ghee

Effect of level of *Piper betel* leaves on physico-chemical qualities of fresh ghee

Moisture

The moisture content in experimental ghee samples was ranged from 0.24-0.25 the minimum was recorded in L_0 (Control) maximum was recorded in L_3 (Table 2 and fig. 5). The effect of treatments was non-significant ($p< 0.05$) in all fresh ghee samples. According to FSSAI (2011) [16] on an average ghee contain fat (99.0 - 99.5%) and moisture (<0.5%). The results obtained are in close agreement with the findings reported by Kumar *et al*. (2016), Sserunjogi *et al.* (1998) [28].

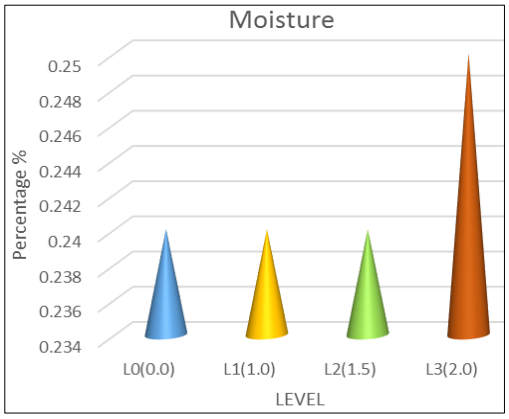

Fig 5: Effect of level of moisture content on ghee

Peroxide value

Peroxide value (PV) is an indicator of extent of primary oxidation production oils and lipids reported by Anwar *et al.* (2007) [3]. peroxide value in *Piper betel* leaves added ghee was 0.00 (NIL) no any reading had recorded in all sample (Table 2 and fig. 6) It means that the level of *Piper betel* leaves exerted a non-significant effect on peroxide value score of ghee. Asha *et al.* (2015) [4] recorded that there was no development of peroxides in treated fresh ghee samples. Patel *et al.* (2012) [23] was observed that during accelerated temperature of ghee storage no rise in a peroxide value and reading was zero in all fresh ghee samples.

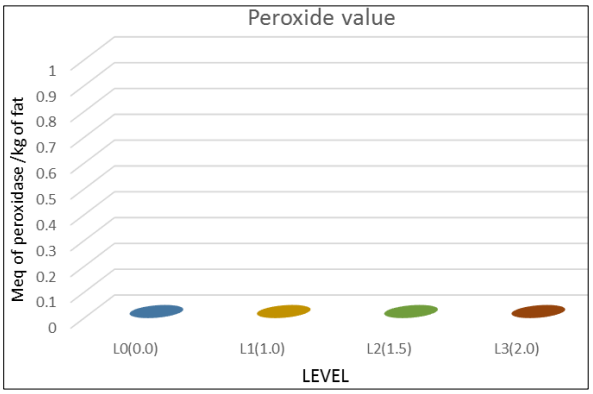

Fig 6: Effect of level of peroxide value content on ghee

Free Fatty Acid

The free fatty acid in *Piper betel* leaves added ghee was (Table 2 and fig. 7) ranged from 0.21 to 0.22 per cent. the level of *Piper betel* leaves exerted a non-significant effect on FFA content of ghee, levels L_0, L_1 and L_2 are at par with each other. Goyal *et al.* (2016) [11] reported that standard value for free fatty acid should not be more than 1.4 per cent. FFA content for fresh cow milk ghee and buffalo milk ghee (without added antioxidant) ranged from average 0.21 to 0.42 (% oleic acid) Gosewade *et al.* (2017) [10].

Table 2: Effect of level of *Piper betel* leaves on Physico chemical properties of fresh ghee

Treatment	Moisture (%)	Peroxide value (meq. of peroxide/kg of fat)	FFA (% of oleic acid)	TBA value (OD)	Conjugate Diene (%)
L_0(0.0)	0.24^a±0.002	Nil	0.21^a±0.005	Nil	0.67^a±0.002
L_1(1.0)	0.24^a±0.004	Nil	0.21^a±0.001	Nil	0.68^b±0.003
L_2(1.5)	0.24^a±0.001	Nil	0.21^a±0.003	Nil	0.68^b±0.005
L_3(2.0)	0.25^a±0.003	Nil	0.22^b±0.001	Nil	0.68^b±0.001
S.Em	0.001	0	0.001	0	0.001
CD(P<0.05)	0.003	0	0.003	0	0.003

*mean ± SE (five)

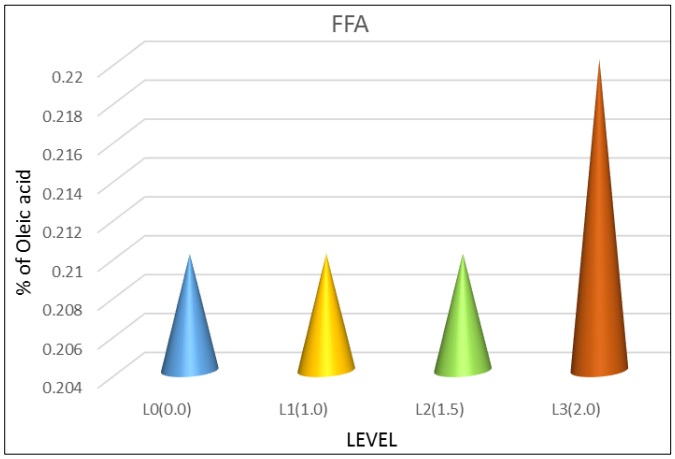

Fig 7: Effect of level of free fatty acid content on ghee

Thiobarbuturic Acid Value

TBA value in *Piper betel* leaves added ghee was 0.00 (NIL) no any reading had recorded in all fresh samples (Table 2 and fig. 8) It means that the level of *Piper betel* leaves exerted a non-significant effect on TBA value score of ghee. initially no rise in a TBA value and reading was zero in all sample. Asha *et al.* (2015) [4] recorded that on a storage of ghee in that zero day there was no development of TBA value in treated ghee sample. Patel *et al.* (2012) [23] was observed that during accelerated temperature of ghee storage no rise in a TBA value and reading was zero in all fresh ghee samples.

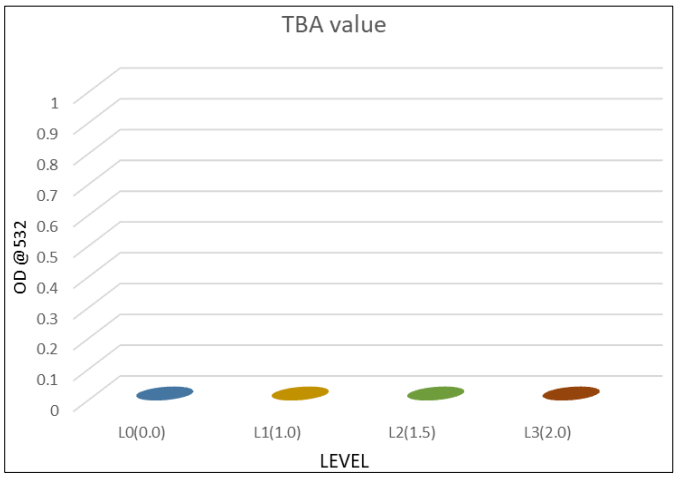

Fig 8: Effect of level of TBA value content on ghee

Conjugated Dienes

Conjugated diene in *Piper betel* leaves added ghee was (Table 2 and fig. 9) ranged from 0.67 to 0.68 percent. Minimum value reported in L_0(Control) 0.67±0.002 and initially the level of *Piper betel* leaves exerted a non-significant effect on conjugated diene of ghee. Pawar *et al.* (2012) [19] reported that conjugated diene reading was 0.6609 for herbal fresh ghee samples later on storage up to 21 days at 80 ºC rise in Conjugated diene up to 1.8 percent and Gosewade observed conjugated diene in fresh buffalo ghee (without added antioxidant) was 0.6670 percent.

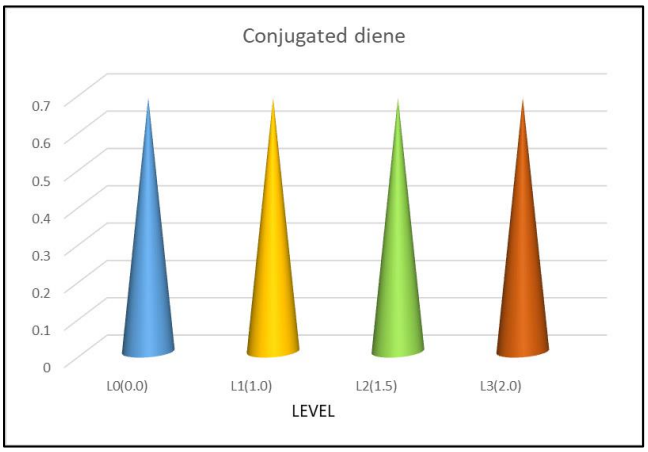

Fig 9: Effect of level of conjugated diene content on ghee

Conclusion

1. The better acceptable *piper betel* leaves added ghee could be prepared by using 1.5 per cent *piper betel* leaves.
2. It was further observed that, level L_2 of fresh ghee containing 1.5 per cent *piper betel* leaves was sensorily superior with higher flavour and colour scores over all other treatments studied.
3. The sensorily best *piper betel* leaves added fresh ghee had the physico chemical properties 0.24% moisture, 0.21% FFA, 0.00 PV, 0.00 TBA and 0.68% conjugated diene.

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