RESEARCH ARTICLE OPEN ACCESS

A Study on Incorporation of Natural Antioxidants For the Shelf Life Extension of Ghee

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ABSTRACT: Natural antioxidants were extracted through aqueous and alcoholic methods from Psidium guajava (guava) leaves and incorporated in ghee for shelf life extension. Total antioxidant content of guava leaves powder, alcoholic and aqueous extract were 51.56. 63.67 and 91.21 respectively. 0.25% and 1% were optimized levels for alcoholic and aqueous extract respectively. optimized alcoholic and aqueous extract treatment samples packed in glass bottles and stored at 60±2ºC for 2 months and evaluated for 10 days interval. Both extract treatments were superior in Peroxide value, TBA, free fatty acids value and RSA then control thus Guava leaves extract could be used as potential natural antioxidants.Guava leaves extracts can be used as potential natural antioxidants to retard auto-oxidation in ghee.

Date of Submission: 04-01-2018 Date of acceptance: 19-01-2018

I. INTRODUCTION

Ghee is considered as the Indian clarified butterfat and as the most important dairy product in human diet. India is the largest milk producer with 155.5 million tonnes per annum and 30-35% of total milk is converting into ghee (NDDB, 2016). Ghee defined as pure clarified fat derived solely from milk or curd or from desi (cooking) butter or from cream to which no colouring matter or preservative has been added (FSSR, 2011). Ghee is chemically composed of complex lipids of triacylglycerol, together with small quantity of free fatty acids, phospholipids, sterols, hydrocarbons, carbonyl compounds, fat soluble vitamins (A, D, E and K), carotenoid pigments, moisture and traces of elements like copper and iron. Ghee contains potential anticarcinogenic compounds such as conjugated linoleic acid (CLA), Sphingomylein, butyric acid and myristic acid. Ghee is being used in Ayurvedic medicine as *ghrita* from ancient times in India. Ghee is utilized as frying and cooking for various food products, garnishing, dressing and spreading over various food products in India.

Low moisture content in ghee determines long shelf life. Oxidation of ghee causes the loss of unsaturated fatty acids, production of objectionable: flavour, destruction of vitamins and carotenes, formation of toxic products, loss of attractive colour and flavour and decrease in nutritive value. BHA (max 200 ppm or 0.02%) is permitted chemical which can be used as antioxidants in ghee (FSSR 2011). But continuous use of synthetic antioxidants may cause health hazards such as teratogenic and carcinogenic effects. Synthetic antioxidants may also cause toxicity of human foods. Ghee has also the potential to assimilate efficiently the properties of ingredients incorporated to it without losing its innate properties.

*Psidium guajava*iscommonly referred as guava of Myrtaceae family and guava leaves contain very high antioxidant activity compared to other herbs. Guava tree bark, fruits, seeds, leaves and roots have been used in indigenous system of medicine for different aliments. *Psidium guajava*leaves are composed of resin, fat, cellulose, tannin, volatile oil, chlorophyll and mineral salts (Nadkarni and Nadkarni 1999). *Psidium guajava*leaves have been used in folk medicine to treat gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions (Morton 1987), it is also revealed that guava leaves exhibit functional properties such as anticarcinogenic, antimicrobial, anti-inflammatory, Antispasmodic and antiviral activity. Considering the above facts, the present study is undertaken to enhance the shelf-life of ghee by incorporating antioxidant extracted from guava leaves with following objectives:To standardise the procedure to incorporate natural antioxidants in ghee, To assess the physico-chemical and sensory parameters of the prepared product and To study the shelf life of the ghee in packaging materials.

II. MATERIALS AND METHODS

Preparation of ghee

Ghee was prepared by direct cream method described by De (2001) with slight modifications.

Extraction of antioxidant compounds from guava leaves

Antioxidant compounds are extracted aqueous and alcoholic extraction methods.

Plant material

Guava leaves were collected in College of Veterinary and Animal Science campus and cleaned with tap water and discarded the unwanted and damaged leaves. The leaves were then air dried for 1 hour to remove the wash water from the surface and Leaves then dried in hot air oven for 48 hours at 50±1ºC. Dried leaves are coarsely grounded guava powder obtained was packed in air tight container and stored at room temperature.

Alcoholic extract

Ethanolic extraction of antioxidant compounds from guava leaves was done according to Baig (2016). The finely ground guava leaves were kept in a 'thimble' of soxhlet chamber and soaked in sufficient ethanol. Extracted alcohol was concentrated by rotary flash evaporator according to Ghorab*et al*. (2007). Evaporated ethnolic solvent was freeze dried and stored at -18ºC.

Aqueous extraction

Aqueous extraction of guava leaves is according to kandil*et al*. (1994) with slight modifications. 100 grams of guava leaves powder is boiled with 1.5 litre of distilled water for 4 hours. Boiling continued until the complete exhaustion of antioxidant compounds. Dissolved compounds are filtered through the whatman paper no 1, 11µm pore size. Filtered solvent with antioxidant compounds is collected in an air tight container and stored at 4ºC. Extracted solvent was concentrated by rotary flash evaporator according to Ghorab*et al*. (2007). Evaporated aqueous solvent was freeze dried and stored at -18ºC.

Free fatty acids (FFA) value

FFA content of ghee samples were determined by standard method as described in manual for analysis of milk and milk products (2015) with slight modifications.

TBA^{rase}(thiobarbituric acid) value

TBA^{rase} value is used for measuring the extent of lipid oxidation. TBA^{rase} value was determined according to manual for analysis of milk and milk products (2015) with slight modifications.

Peroxide Value

It was estimated to measure the resistance of ghee to oxidation and expressed in terms of days for which it remains good for consumption. The Peroxide value of ghee was determined by following manual for analysis of milk and milk products (2015) with slight modifications.

Radical scavenging activity in ghee

Radical scavenging activity in ghee incorporated with natural antioxidants was determined by keeping the samples at 80±1ºC for 14 days in a hot air oven. Ghee samples were packed in glass bottles. The capacity of antioxidants to quench DPPH radical in ghee was determined before and after accelerated oxidation tests (Espin*et. al*., 2000). Ethyl acetate was used as a better solvent for hydrophobic compounds.

III. Results

Quantitative assessment of antioxidants in extracts

Total antioxidants were analysed by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay for guava leaves powder, aqueous extract and alcoholic extract. Among three, alcoholic extract showed more antioxidant properties compared to others.

Table 1: Total antioxidant activity of extracts

Type of Extract	Total antioxidants (in %)
Leaves powder	51.56
Aqueous extract	63.67
Alcoholic extract	91.21

Optimization of extract

Alcoholic extract was added at four various levels viz. 0.25% (T2), 0.5% (T3), 0.75% (T4) and 1% (T5) to ghee. These treatments were compared with control treatment (T1) ghee prepared by direct cream method. Aqueous extract was added at four various levels viz. 0.25% (T7), 0.5% (T8), 0.75% (T9) and 1% (T10) to ghee. These treatments were compared with control treatment (T6) ghee prepared by direct cream method. The results were analyzed statistically using SPSS software. Results were taken for four replications.

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**	**Chi-square value**
Flavour	48.15±0.12^{a}	44.25±0.17^{ab}	37.15±0.34^{b}	35.20±0.14^{b}	33.60±0.14^{bc}	18.327**
Body and texture	28.05±0.15^{a}	27.25±0.17^{ab}	25.95±0.15^{b}	25.05±0.15^{b}	24.55±0.12^{bc}	18.167**
colour	8.30±0.12^{a}	7.15±0.05	5.15±0.0	4.40±0.08^{b}	4.05±0.22^{b}	17.707**

		[a]	9[ab]			
Free from suspended solids	9.80±0.08[a]	9.70±0.05[a]	9.65±0.05[a]	9.55±0.05[a]	9.50±0.05[a]	9.745*
Overall acceptability	92.25±0.17[a]	85.50±0.34[ab]	80.35±0.30[b]	76.05±0.22[b]	72.25±0.17[bc]	18.286**

Figures are the Mean± Standard error of four replications, **-significant at one percent level ($p<0.01$), [a-c]figures in row bearing different superscripts differ significantly, *-significant at one percent level ($p<0.05$)

Table 2: Effect of different levels of alcoholic extract on sensory characteristics of ghee

Parameter	T6	T7	T8	T9	T10	Chi- square value
Flavour	47.80±0.18[a]	44.05±0.22[a]	43.70±0.20[a]	43.65±0.17[a]	43.60±0.14[a]	10.971*
Body and texture	28.05±0.15[a]	27.65±0.05[a]	27.55±0.05[a]	27.45±0.12[a]	27.65±0.22[a]	8.003[ns]
colour	8.45±0.09[a]	8.20±0.08[a]	8.15±0.05[a]	8.05±0.05[a]	8.10±0.12[a]	8.023[ns]
Free from suspended solids	9.85±0.05[a]	9.750±0.05[a]	9.70±0.05[a]	9.65±0.05[a]	9.60±0.08[a]	7.703[ns]
Overall acceptability	93.60±0.37[a]	88.50±0.28[a]	88.25±0.21[a]	88.15±0.09[a]	88.05±0.20[a]	10.946*

Figures are the Mean± Standard error of four replications, *-significant at one percent level ($p<0.05$), [a]figures in row bearing different superscripts differ significantly, ns-non-significant

Table 3: Effect of different levels of aqueous extract on sensory characteristics of ghee

Shelf Life Study Of Ghee Incorporated With Natural Antioxidants

Ghee prepared with optimized level of both aqueous extract and alcoholic extract were packed in glass bottles, polypropylene cups and PET bottles and stored at 60±2ºC for two months. The chemical parameters adjudged are peroxide value, FFA value and TBA value. Chemical parameters were observed for every 10 days period of interval. All the treatment results were taken for 6 replications. The variation between different periods of measurements related to peroxide value, FFA value and TBA valuewas observed using repeated measures of ANOVA and variation between control sample and treatment sample for every period was analyzed by independent t-test.

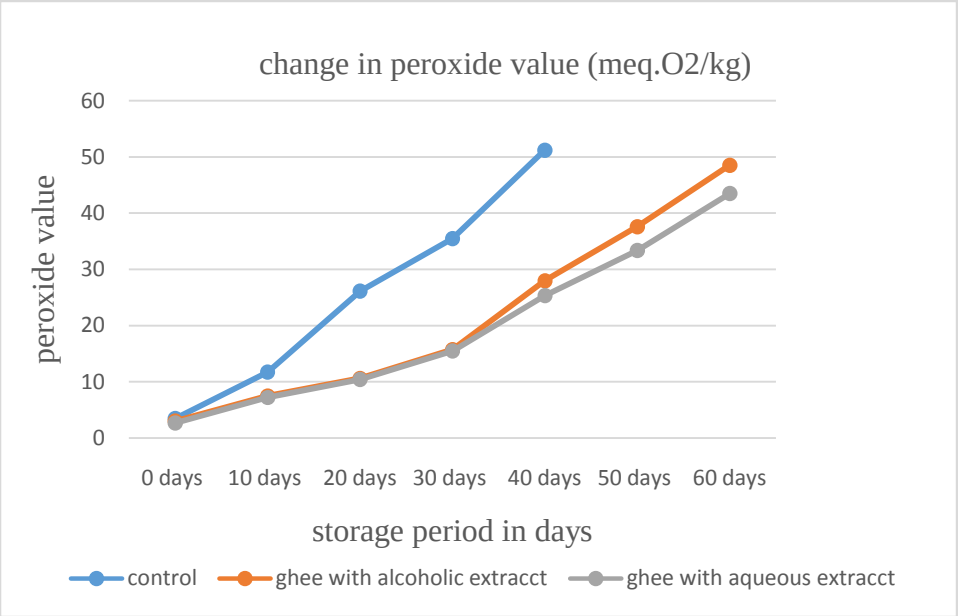

Fig 1: Change in peroxide value in shelf life study

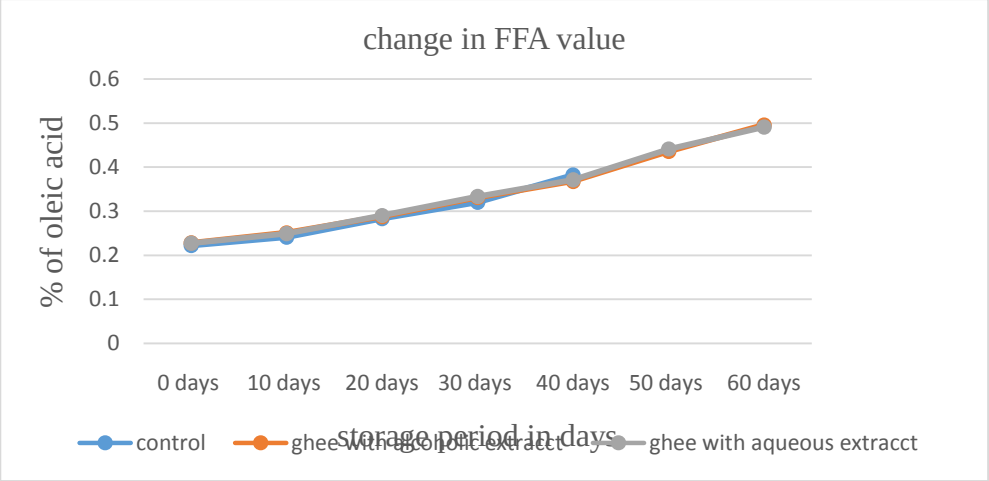

Fig 2: Change in FFA in shelf life study

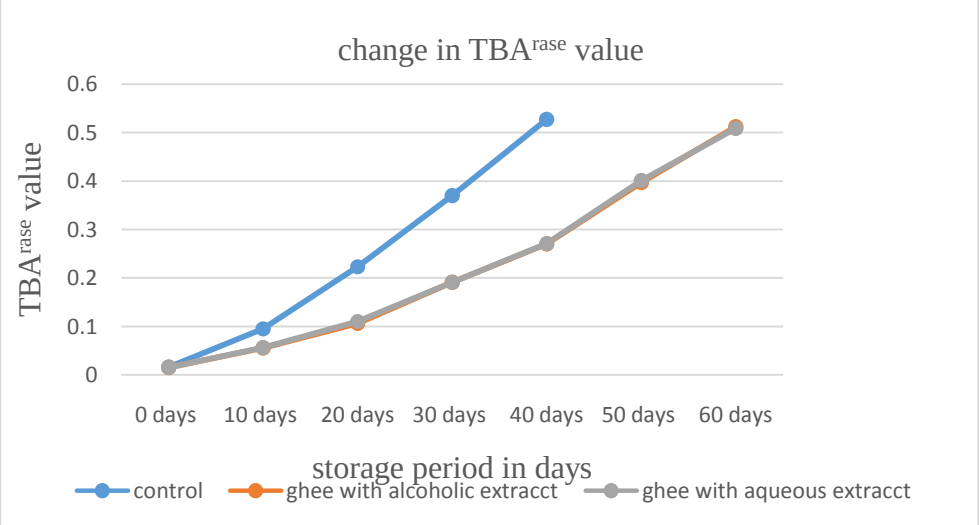

Fig 16: Change in TBA^{rase} value when samples packed in glass bottles

Effect of shelf study on change in radical scavenging activity (DPPH assay)

Ghee prepared with optimized level of both aqueous extract and alcoholic extract were packed in glass bottles and stored at 80±2ºC for 14 days. The changes in radical scavenging activity determined by DPPH assay was observed for every 2 days period of interval for 14 days. The variation between different periods of measurementswas observed statistically.

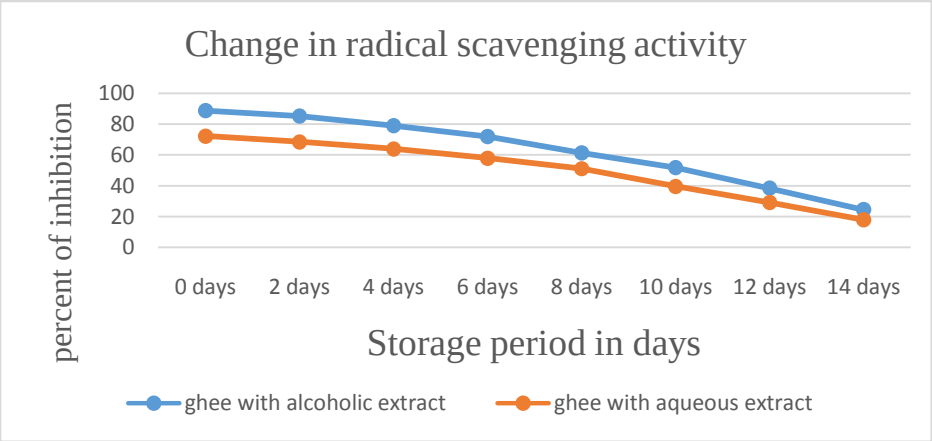

Fig 4: Change in radical scavenging activity in storage study

IV. DISCUSSION

Assessment of total antioxidant activity

The results for total antioxidant activity of guava leaves powder, aqueous extract and alcoholic extract are presented in table 1 and the total percentage antioxidant activity was 51.56, 63.67 and 91.21 respectively.

Optimization of extract

Optimization of extract

Optimization of alcoholic extract was done by the sensory evaluation of ghee prepared with various levels of freeze dried alcoholic extract is presented in Table 2. There was no significant difference between control treatment that is T1 and 0.25% level that is T2 in flavour, body and texture, colour, free from suspended solids and overall acceptability. There was significant difference between T1 and other three treatments like T3, T4 and T5 in falvour, body and texture, colour and overall acceptability. There was no significant difference between any treatments in free from suspended solids. Treatment T2 was considered as the optimized level of alcoholic extracts which showed relatively similar sensory parameters as that of control.

Optimization of alcoholic extract was done by the sensory evaluation of ghee prepared with various levels of aqueous extract of guava leaves for optimization of level of extract is presented in table 7. There was no significant difference between treatments T6, T7, T8, T9 and T10 in flavour, body and texture, colour, free from suspended solids and overall acceptability. T10 treatment that is 1% level of aqueous extract showed more acceptable colour scores and was selected as optimized level of aqueous extract.

Shelf life study of ghee incorporated with natural antioxidants

change in peroxide value

The effect of storage period on change in peroxide value for control, alcoholic and aqueous extract treatment ghee samples are explained in figure 1. There was no significant ($p>0.05$) difference between control sample and alcoholic extract treatment samples and between control and aqueous extract treatment samples in Peroxide values on 0^{th} day. There was significant difference ($p<0.01$) between intervals for 40 days of storage in control samples. There was significant difference ($p<0.01$) between the intervals of alcoholic and aqueous extract treatment samples for 60 days of storage. The change in Peroxide values of control, alcoholic and aqueous extract treatment samples are 3.46 to 51.20, 2.93 to 48.53 and 2.66 to 43.46 respectively. alcoholic extract and aqueous extract were effective against formation of peroxides in ghee.

These results were found to be in agreement with the results of Siddiq *et al.*, (2005) whoreported that development of peroxides were significantly constrained by the addition of methanolic (80 and 100%) and acetone (80 and100%) extracts of *Moringa oleifera*to sunflower oilunder accelerated conditions. Among differentmethanolic and acetone extracts, 80% methanolic extract showed prominent retardation in peroxide value than other methanolic and acetone extracts.

Change in FFA value

The effect of storage period on change in FFA value for control, alcoholic and aqueous extract treatment ghee samples are enlightened in figure 2. There was significant ($p<0.05$) difference between

control sample and alcoholic extract treatment samples and between control and aqueous extract treatment samples in FFA value on 0^{th} day. There was significant difference ($p<0.01$) between intervals for 40 days of storage in control samples. There was significant difference ($p<0.01$) between the intervals of alcoholic and aqueous extract treatment samples for 60 days of storage. The change in FFA value of control, alcoholic and aqueous extract treatment samples were 0.22 to 0.38, 0.22 to 0.49 and 0.22 to 0.44% of oleic acid respectively.

Patel and Rajorhia (1979) reported that after 30 days of storage, a progressive increase in free fatty acid content was observed in all ghee samples, the increase in free fatty acidity was parallel to development of peroxide value, the controlsamples of ghee after 147 day of storage at30˚Cshowed increase in FFA by more than 100percent. Betel leaves at 1.0 per cent concentration provided maximumprotection against the hydrolysis of ghee. The effectivenessagainst hydrolysis decreased in the order: curry leaves at 1 percent, betel leaves 1 percent, BHA+BHT at 0.02 per cent, betel leaves at0.5 percent, curry leaves at 0.8 per cent, curry leaves 0.5% percent and betelleaves at 0.2 per cent concentration.

Change in TBA^{rase}(Thiobarbituric acid)value

The effect of storage period on change in TBA^{rase} value for control, alcoholic and aqueous extract treatment ghee samples are elucidated in figure 3. There was no significant ($p>0.05$) difference between control sample and alcoholic extract treatment samples and between control and aqueous extract treatment samples in TBA^{rase} value on 0^{th} day. There was significant difference ($p<0.01$) between intervals for 40 days of storage in control samples. There was significant difference ($p<0.01$) between all the intervals of alcoholic and aqueous extract treatment samples for 60 days of storage. The change in TBA^{rase} value control, alcoholic and aqueous extract treatment samples were 0.01 to 0.52, 0.01 to 0.51 and 0.01 to 0.50 respectively. alcoholic and aqueous extracts were reduced the development of TBA^{rase}that of control sample.

The results are in argument with Shourbagyand El-Zahar (2014) with increase in TBA^{rase} in control treatment than peanut skins, pomegranate peelsand olive pomace ethanol (80%) extract when samples are stored at 63˚C for 21 days. It is notable that peanut skins extracts exhibited strong antioxidantcapacity in all assays used followed by pomegranate peelsand olive pomace extracts.Theseextract were added at a rate of 200, 400 and 600ppm. peanut skins extract exhibited same inhibition of TBA^{rase}as ghee added with 200ppm BHA.

Effect of shelf study on change in radical scavenging activity (DPPH assay)

The effect of storage period on change in radical scavenging activity for optimized alcoholic and aqueous extract treatment ghee samples are elucidated in figure 4.There was significant ($p<0.01$) difference alcoholic extract treatment samples and aqueous extract treatment samples in RSA value on 0^{th} day. There was significant difference ($p<0.01$) between all the intervals of alcoholic and aqueous extract treatment samples for 14 days of storage. The change in radical scavenging activity (% of inhibition) value foralcoholic and aqueous extract treatment samples were 88.83 to 24.47 and 72.34 to 17.93 respectively. alcoholic and aqueous extracts of guava leaves are highly effective in breaking the radical formation reaction to inhibit the autooxidation.

A study conducted by Pawar*et al.,* (2012) reported that The RSA in DPPH system of shatavari extract (aqueous and ethanolic), rosemary andgreen tea extract, as wellas BHA and TBHQ, was evaluated at200 ppm. Ethanolic extract of shatavari exhibited significantly ($P < 0.05$) higher RSA than aqueous extract. However, activity exhibited by both the extractsof shatavari was significantly ($P < 0.05$) lower than that of thenatural (rosemary and green tea) and synthetic antioxidants(TBHQ and BHA). Parmar *et al.,* (2013) stated that ethanolic extract of Arjuna has significant ($P<0.05$) radical scavenging ability of ghee and this was more pronounced in case of cow ghee than in buffalo ghee.

V. CONCLUSION

It could be concluded that ethanolic extract had high antioxidant activity compared to aqueous extract and guava leaves powder. Alcoholic extract at 0.25 per cent and aqueous extract of 1 percent effectively retarded the auto-oxidation in ghee. Aqueous extract ghee was superior quality in terms of sensory but antioxidant potential wise alcoholic extract was more effective than aqueous extract. The shelf life of both alcoholic and aqueous extract treatment was 60 days where control sample was 4o days at elevated temperature of 60±2°C.the findings suggested that Psidium guajava leaves alcoholic and aqueous extracts could be used as natural antioxidants. Freshly prepared ghee with guava leaves extract can be used to prevent free radical related disorders.

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Aditya N.,Divya M.P. "A Study on Incorporation of Natural Antioxidants For the Shelf Life Extension of Ghee." International Journal of Engineering Research and Applications (IJERA), vol. 08, no. 01, 2018, pp. 10–16.