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RESEARCH ARTICLE

Antioxidant property of palm oil blended ghee and its fractions

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Abstract: Ghee is a high-priced product that costs three to six times than the edible vegetable oil. Dry fractionation is one of the cost-effective methods of modifying the physical properties of milk fat. Natural antioxidant present in palm oil is believed to enhance the oxidative stability of ghee. Therefore, in the current study, ghee was intentionally blended with palm oil at levels of 0, 5, 10, and 20%. Then the ghee was fractionated to separate liquid and solid portions by dry fractionation technique. The antioxidant activity accessed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method shows that as the palm oil (especially tocotrienols and tocopherols) level increased the antioxidant activity in the prepared samples. This could be due to carotenoids and vitamins E which act synergistically as powerful natural antioxidants which also contribute better towards stability against auto oxidation (peroxide value). The liquid fraction (L_{20} with palm oil 20%) has a high level of antioxidant activity. Fractionated ghee, especially the liquid fraction showed higher antioxidant activity as compared to ghee could be due to its higher vitamin E activity. The peroxide value and free fatty acid content of ghee were determined during storage at 30°C and 60°C for 90 days. Liquid fractions showed more peroxide value and FFA as the storage day increased than solid fractions, which contained more peroxide value and FFA than control samples.

Keyword: - Antioxidant activity; Free fatty acid; Ghee; Palm oil; Peroxide value

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Introduction

Ghee is also known as clarified butterfat. It has a pleasant and appetizing aroma and is prepared from cream or butter made from cow or buffalo milk, or a combination of these two. Ghee is most widely used milk product in the Indian subcontinent and is regarded as the best cooking and frying medium. During storage, ghee undergoes oxidative degradation, resulting in changes to major quality parameters such as intensity of colour decrease as the palm oil level increases (0, 5, 10 and 20 per cent), flavour, aroma and nutritive value, affecting its suitability for consumption. The development of rancidity reduces the shelf life of the product, which ultimately affects consumer acceptability. Various studies have found that oxidized lipids may be harmful to one's health (Nerin et al. 2008). Since, India is the primary producer and exporter of Ghee, it is a one of the costliest dairy product in India (Ghee Market in India: Industry Trends 2022). The acceptability of ghee is largely determined by the extent of oxidative deterioration. Several chemical methods have been developed to measure the oxidative changes in oils and fats (Gray, 1978). The methods reported for monitoring the oxidative deterioration of various oils and fats are based on chemical changes that occur at different stages of oxidation, namely the primary and secondary stages. Peroxides, particularly hydroperoxides, are the first compounds formed during oxidation and are known as primary oxidation products. Peroxide value (PV) is most commonly used as an indicator of the early stages of oxidation in fats and oils. Oxidative stability is an important factor in oil quality, and is particularly significant for oils used for frying because of the high temperature and long duration of the frying process. The chemistry of oxidation at high temperatures is very complex since both thermal and oxidative reactions are involved (Marquez-Ruiz and Dobarganes, 2007). Vegetable oils such as palm oil, olive oil, cottonseed oil, peanut oil, and sunflower oil are classed as Oleic – Linoleic acid oils seeing that they contain a relatively high proportion of unsaturated fatty acids (monounsaturated fatty acid like oleic acid and the polyunsaturated fatty acid like linoleic acid) (Dunn, 2005; Gertz et al. 2000). Palm oil is edible plant oil extracted from palm tree fruits. Palm oil (*Elaeis guineensis*) is extracted from the pulp of the palm fruit (Poku, 2002). Palm oil has been used in food preparation for over 5,000 years. Palm oil is the most widely

produced edible vegetable oil in the world, and its nutritional and health benefits have been well documented (Chandrasekharan et al. 2000). The most abundant natural source of tocotrienol is palm oil. Because of its good resistance to oxidative deterioration and better ability to withstand the high temperatures used in frying than other oils, the industrialization of convenience foods and snack food manufacturing opened up additional uses for palm oil (Kheri, 1987; Berger, 1992). Crude palm oil (CPO, also known as red palm oil, RPO) contains both healthy beneficial compounds like vitamin, carotenoids and phytosterols as well as impurities like phospholipids, free fatty acids (FFA), gums and lipid oxidation products, which can be removed through refining processes which causes off-flavour/ odour to oil (Sambanthamurthi et al. 2002). Centrifugation and drying are commonly used to purify the CPO. After purification oil is cooled and kept in a suitable container. CPO is the highest natural source of carotenoids (500–700 ppm), tocopherols (600–1200 ppm), and tocotrienols (600–1200 ppm), all of which contribute to its nutritional value and oxidative stability. Their antioxidant effects, which are primarily directed against reactive oxygen species (ROS), play a role in the prevention of ageing, CVD and cancer. Tocotrienols are natural cholesterol production inhibitors (Edem, 2002). The carotenoids and the vitamins E act synergistically as powerful natural antioxidants. Palm oil is largely made up of unsaturated fatty acids, which can raise blood cholesterol levels as compare to other oil. (Edem, 2002). In the present investigation, the ghee was blended with palm oil (at concentration 0, 5, 10, and 20%.) and fractionated by dry fractionation technique. Antioxidation activity along with FFA and peroxide value from palm oil blended ghee and its fractions.

Materials and methods

Butter was purchased from retail outlet of local market, Bengaluru. Palm oil was purchased from the local Bengaluru market and used for the adulteration purpose.

Preparation of samples

The creamery butter method was used to produce ghee (Parmar and Khamrui, 2017). It is then clarified in a stainless steel vessel over direct flame into ghee with continous strring at a temperature of 115-117C. Ghee was then filtered through muslin cloths, cooled and filled in airtight glass bottles for futher analysis.

Fatty acid profile of cow milk fat

Fatty acid	Cow milk fat (%)
Butyric acid (C4:0)	1.78
Caproic acid (C6:0)	1.44
Caprylic acid (C8:0)	0.99
Capric acid (C10:0)	2.55
Lauric acid (C12:0)	3.15
Myristic acid (C14:0)	10.30
Palmitic acid (C16:0)	24.03
Palmitoleic acid (C16:1)	1.18
Stearic acid (C18:0)	9.36
Oleic acid (C18:1)	20.04
Linoleic acid (C18:2)	1.64
Linolenic acid (C18:3)	0.66
Arachidonic acid (C20:4)	0.11

(Pena-Serna and Restrepo-Betancur, 2020)

Fatty acid profile of palm oil

Fatty acid	Palm oil (%)
Lauric	0.19
Myristic	1.10
Palmitic	46.38
Stearic	4.6
Oleic	38.08
Linoleic	9.33
Linolenic	-
Arachidonic	0.38

(Li et al. 2011)

Dry fractionation technique

Fractionation was done by following Kankare (1974) method with slight modification in temperature and time period of centrifugation. Melting method was used to fractionate ghee into solid and liquid fractions. The crystal nuclei memory was removed by heating ghee to 60°C. It was then progressively cooled to 30°C in an incubator for 12 h to crystallize. After centrifugation (at 2500 rpm for 15 min) using temperature-controlled centrifuge at 30°C, the liquid was separated from the crystals by decantation. At 30°C, solid fraction obtained (S_{30}) was considered as a high melting fraction. The liquid fraction collected at 30°C was then incubated at 20°C for 12 h. Again, the fraction was centrifuged at 20°C under same conditions. The produced crystals were separated. The solid portion obtained at 20°C (S_{20}) was considered a medium melting fraction, whereas the amount that remained liquid at 20°C was referred to as the low melting fraction (L_{20}).

Antioxidant Activity

The percentage of antioxidant activity (AA %) of each fraction (S_{20} and L_{20}) of ghee, control ghee as well as palm oil was assessed by DPPH free radical assay as described by Wu et al. (2006).

FFA and Peroxide

The FFA content was determined as per SP: 18 (Part XI)-(1981). The peroxide value was determined by method as described in AOAC (2000). The prepared ghee samples were stored in 30 ºC for 3 months and accelerated storage at 60 ºC for 3 months.

Statistical analysis

Significant difference between the values was verified by one way analysis of variance (ANOVA) and comparison between means was made by critical difference value by using R software [R. version 4.1.2 (2021-11-01), copyright © 2021, R foundation].

Results and discussion

The antioxidant activity of palm oil was found to be 92.5 %, respectively. The antioxidant activity in ghee added with palm oil (0, 5, 10 and 20 %) measured by DPPH method is shown in Table 1. Ghee with palm oil (20 %) had higher antioxidant activity as compared to other ghee sample. The statistical analysis reveals that significant ($P \leq 0.05$) difference amongst ghee sample with respect to antioxidant activity. The antioxidant activities in liquid and solid fractions of ghee added with palm oil (0, 5, 10 and 20 %) are shown in the Table 1. L_{20} with palm oil (20 %) had higher antioxidant activity as compared to other ghee samples. It was evident from the Table 1 that significant ($P \leq 0.05$) difference amongst liquid fraction of ghee sample respect to the antioxidant activity. Fraction S_{20} with palm oil (20 %) had higher antioxidant activity as compared to other ghee samples. Statistically it was proved that significant ($P \leq 0.05$) difference amongst solid fraction of ghee sample respect to antioxidant activity.

S_{20} ($P \leq 0.05$)

The prepared ghee samples were stored in 30 ºC for 3 months and accelerated storage at 60 ºC for 3 months. The samples were analysed for storage stability viz. hydrolytic (per cent FFA) and oxidative rancidity (Peroxide value). FFA and peroxide value were determined periodically at an interval of 30 days as per ISI (1981) for a period of 90 days.The peroxide value of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0 mM O_2/kg of fat, respectively in all the ghee sample at 0^{th} day and it increased to 2.67, 2.45, 2.15 and 1.89 mM O_2/kg of fat, respectively for ghee blended with 0, 5, 10 and 20 percent of palm oil on 90^{th} day of storage at 30°C (Table 2). The peroxide value in liquid fraction of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0 m.MO_2/kg of fat, respectively in all the sample at 0^{th} day and it was increased to 4.87, 4.57, 3.87 and 3.63 mM O_2/kg of fat, respectively on 90^{th} day of storage at 30°C (Table 2). The peroxide value in solid fraction of ghee added with palm oil (0, 5, 10 and 20 %) were found to be 0 mM O_2/kg of fat, respectively in all the

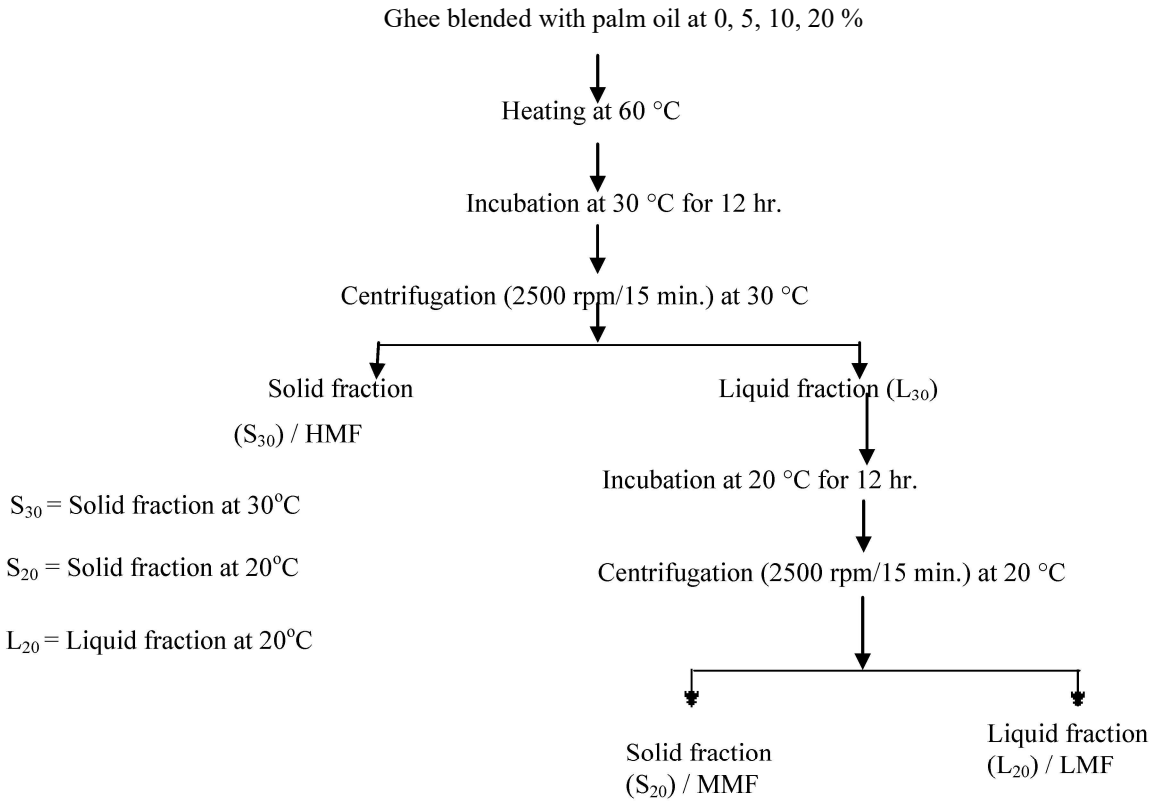

Fig: 1: Dry fractionation technique

sample at 0th day and it increased to 3.77, 3.20, 2.96 and 2.34 mM O_2/kg of fat, respectively on 90th day of storage at 30°C (Table 2).

The peroxide value in ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0 mM O_2/kg of fat, respectively in the entire sample at 0th day and it increased to 8.01, 7.35, 6.45 and 5.67 mM O_2/kg of fat, respectively on 90th day of storage at 60°C (Table 2). The peroxide value in liquid fraction of ghee added with palm oil (0, 5, 10 and 20 %) were found to be 0 mM O_2/kg of fat, respectively in all the samples at 0th day and it increased to 14.61, 13.71, 11.61 and 10.89 mM O_2/kg of fat, respectively on 90th day of storage at 60°C (Table 2). The peroxide value in solid fraction (S_{20}) of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0 mM O_2/ kg of fat, respectively in all the

Table 1 Antioxidant activity of ghee, liquid fraction and solid fraction sample added with palm oil

Sample	Antioxidant activity (%)	Sample	Antioxidant activity (%)	Sample	Antioxidant activity (%)
Palm oil	92.5[a]	L20 (Control)	55.1[d]	S20 (Control)	49.9[d]
Control ghee	62.9[e]				
Ghee with PO (5%)	68.3[d]	L20 with PO (5%)	71.3[c]	S20 with PO (5%)	56.1[c]
Ghee with PO (10%)	77.3[c]	L20 with PO (10%)	84.8[b]	S20 with PO (10%)	58.2[b]
Ghee with PO (20%)	83.1[b]	L20 with PO (20%)	86.4[a]	S20 with PO (20%)	65.3[a]
CD (*P≤0.05*)	0.55	CD (*P≤0.05*)	0.56	CD (*P≤0.05*)	0.50

CD-Critical difference
All the values are average of three trials
Different superscripts within the column are compared with other values

Table 2: Effect of storage on peroxide value of ghee, liquid fraction of ghee and solid fraction of ghee added with palm oil stored at 30 ºC and 60 ºC

Sample	Peroxide value (m.M O2/Kg of fat)							
	Storage period (in Days) 30 ºC				Storage period (in Days) 60 ºC			
Days	0th	30th	60th	90th	0th	30th	60th	90th
Control ghee	0	0.52[a]	1.48[a]	2.67[a]	0	1.56[a]	4.44[a]	8.01[a]
Ghee with PO(5%)	0	0.50[b]	0.85[b]	2.45[b]	0	1.50[b]	2.55[b]	7.35[b]
Ghee with PO(10%)	0	0.47[c]	0.54[c]	2.15[c]		1.41[c]	1.62[c]	6.45[c]
Ghee with PO(20%)	0	0.42[d]	0.50[d]	1.89[d]	0	1.26[d]	1.50[d]	5.67[d]
CD (P≤0.05)	NS	0.01	0.01	0.01	NS	0.01	0.55	0.01
L20 (Control)	0	0.78[a]	3.53[a]	4.87[a]	0	2.34[a]	10.6[a]	14.61[a]
L20 with PO(5%)	0	0.67[b]	3.00[b]	4.57[b]	0	2.01[b]	9.01[b]	13.71[b]
L20 with PO(10%)	0	0.58[c]	2.49[c]	3.87[c]	0	1.74[c]	7.47[c]	11.61[c]
L20 with PO(20%)	0	0.52[d]	1.89[d]	3.63[d]	0	1.56[d]	5.67[d]	10.89[d]
CD (*P≤0.05*)	NS	0.01	0.01	0.01	NS	0.01	0.52	0.74
S20 (Control)	0	0.65[a]	2.56[a]	3.77[a]	0	1.95[a]	7.68[a]	11.31[a]
S20 with PO (5%)	0	0.55[b]	2.00[b]	3.20[b]	0	1.65[b]	6.01[b]	9.60[b]
S20 with PO (10%)	0	0.49[c]	1.86[c]	2.96[c]	0	1.47[c]	5.58[c]	8.88[c]
S20 with PO (20%)	0	0.45[d]	1.59[d]	2.34[d]	0	1.35[d]	4.77[d]	7.02[d]
CD (P≤0.05)	NS	0.01	0.01	0.01	NS	0.01	0.6	0.57

CD-Critical difference
All the values are average of three trials
Different superscripts within the column are compared with other value

sample at 0th day and it was increased to 11.31, 9.60, 8.88 and 7.02 mM O_2/kg of fat, respectively on 90th day of storage at 60°C (Table 2).

The FFA content in ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0.32, 0.68, 1.00 and 1.70 percent oleic acid (O.A), respectively at 0th day and it was increased to 0.69, 0.92, 1.53 and 2.15 per cent O.A, respectively on 90th day of storage at 30°C (Table 3). The FFA content in liquid fraction of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0.34, 0.70, 1.20 and 1.90 per cent O.A, respectively at 0th day and it were increased to 0.89, 0.96, 1.75 and 2.34 per cent O.A, respectively on 90th day of storage at 30°C (Table 3).The FFA content in solid fraction of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0.33, 0.69, 1.10 and 1.80 per cent O.A, respectively at 0th day and it was increased to 0.77, 0.95, 1.59 and 2.19 per cent O.A, respectively on 90th day of storage at 30°C (Table 3).

The FFA content of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0.32, 0.68, 1.00 and1.70 per cent O.A, respectively at 0th day and it was increased to 0.69, 0.92, 1.53 and 2.15 per cent O.A, respectively on 90th day of storage at 60°C (Table 3).The FFA in liquid fraction of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0.34, 0.70, 1.20 and 1.90 per cent O.A, respectively at 0th day and it was increased to 2.67, 2.88, 5.25 and 7.02 per cent O.A, respectively on 90th day of storage at 60°C (Table 3).The FFA content in solid fractions of ghee added with palm oil (0, 5, 10 and 20 per cent) were found to be 0.33, 0.69, 1.10 and 1.80 per cent O.A, respectively at 0th day and it was increased to 2.31, 2.85, 4.77 and 6.57 per cent O.A, respectively for 0, 5, 10 and 20 per cent samples on 90th day of storage at 60°C (Table 3).

The assay involved 50 mg/ 100 ml (ethanol) DPPH solution, leading to the detection of even up to 5% level of palm oil adulteration in ghee. Ramani et al. (2019) described the effect of a chromogenic analytical method for detecting palm oil adulteration in ghee. Palm oil was added to ghee in quantities of 5, 10, 15, and 20%. The detection limits were approximately 5%. (activity 68.3) The method proposed proved to be simple and appropriate for the detection of palm oil in ghee even at 5% level. In the current study L_{20} fraction with PO (20%) have showed highest antioxidant activities (86.4 %) as compared to control ghee (activity 55.1%). This could be due to the presence carotenoids and vitamins E which acts synergistically as powerful natural antioxidants and also provides better stability toward auto oxidation (Mba et al. 2015). The results obtained was comparable with those of Alyaqoubi et al. (2014), reported that anti-oxidant activity of ghee was 60.81 % by DPPH method. Similar results were also found by Ramani et al. (2018) reported that DPPH reaction (qualitative) of pure ghee and ghee adulterated with palm oil (5, 10, 15 and 20 %). During storage, as the palm oil level increased the antioxidant activity also increased. Therefore, it could be concluded that the carotenoids and the vitamins E acts as an strong antioxidants and hence enhances the shelf life of the ghee.

Table 3: Effect of storage on FFA value in ghee, liquid fraction of ghee and solid fraction of ghee added with palm oil stored at 30 ºC and 60ºC

Sample	FFA (% OA)							
	Storage period (in Days) 30ºC				Storage period (in Days) 60ºC			
Days	0th	30th	60th	90th	0th	30th	60th	90th
Control ghee	0.32^{d}	0.45^{d}	0.58^{d}	0.69^{d}	0.32^{d}	1.35^{d}	1.74^{d}	2.07^{d}
Ghee with PO(5%)	0.68^{c}	0.74^{c}	0.83^{c}	0.92^{c}	0.68^{c}	2.22^{c}	2.49^{c}	2.76^{c}
Ghee with PO(10%)	1.00^{b}	1.20^{b}	1.38^{b}	1.53^{b}	1.00^{b}	3.60^{b}	4.14^{b}	4.59^{b}
Ghee with PO(20%)	1.70^{a}	1.90^{a}	2.05^{a}	2.15^{a}	1.70^{a}	5.70^{a}	6.15^{a}	6.45^{a}
CD (*P≤0.05*)	0.09	0.01	0.01	0.01	0.09	0.52	0.47	0.55
L20 (Control)	0.34^{d}	0.70^{d}	0.78^{d}	0.89^{b}	0.34^{d}	2.10^{d}	2.34^{c}	2.67^{c}
L20 with PO(5%)	0.70^{c}	0.82^{c}	0.89^{c}	0.96^{b}	0.70^{c}	2.46^{c}	2.67^{c}	2.88^{c}
L20 with PO(10%)	1.20^{b}	1.50^{b}	1.66^{b}	1.75^{a}	1.20^{b}	4.50^{b}	4.98^{b}	5.25^{b}
L20 with PO(20%)	1.90^{a}	2.04^{a}	2.27^{a}	2.34^{a}	1.90^{a}	6.12^{a}	6.81^{a}	7.02^{a}
CD (*P≤0.05*)	0.01	0.01	0.01	0.74	0.01	0.01	0.76	0.58
S20 (Control)	0.33^{d}	0.55^{d}	0.70^{d}	0.77^{a}	0.33^{d}	1.65^{d}	2.10^{d}	2.31^{c}
S20 with PO (5%)	0.69^{c}	0.78^{c}	0.86^{c}	0.95^{a}	0.69^{c}	2.34^{c}	2.58^{c}	2.85^{c}
S20 with PO (10%)	1.10^{b}	1.34^{b}	1.44^{b}	1.59^{a}	1.10^{b}	4.02^{b}	4.32^{b}	4.77^{b}
S20 with PO (20%)	1.80^{a}	1.95^{a}	2.10^{a}	2.19^{a}	1.80^{a}	5.85^{a}	6.30^{a}	6.57^{a}
CD (P≤0.05)	0.33^{d}	0.01	0.01	0.65	0.09	0.50	0.38	0.68

CD-Critical difference
All the values are average of three trials
Different superscripts within the column are compared with other value

Peroxide value in ghee was decreased with increasing levels of palm oil (5, 10, 20 % respectively). It confirms with work carried out by Niranjan, (2017) that the control ghee sample had a peroxide value of 1.4 mM O_2/Kg of fat on 15th day and then it raised drastically to a peroxide value of 6 mM O_2/Kg of fat on 25th day of storage. Similar results were also found by Archana, (2019) report that ghee stored at 29 °C for 0 day to 60 days had a peroxide value of 0 to 1.50 mM O_2/Kg. This is probably due to the unsaturated fatty acids in fat are oxidised, and the principal oxidation products are hydroperoxides. Though peroxides are not responsible for the development of off-flavours in and of themselves, their measurement provides a good indication of the degree of auto-oxidation. Several elements that determine the quality of ghee have been reported to alter the rate of auto-oxidation. Ghee prepared using the desi method developed peroxides more quickly than creamery ghee (Lalitha and Dastur, 1983). When ghee was clarified at a higher temperature (120 °C), it produced peroxide at a much slower rate than when it were clarified at a lower temperature (Narayanan et al. 1996). Other elements that affect the keeping quality of ghee include storage temperature, antioxidants, metal contamination, dissolved oxygen, and light exposure. It confirms with work carried out by Niranjan, (2017) The desi ghee samples exhibited a peroxide value ranging from 1.4 to 1.7 mM.O_2 /kg fat on 15th day of storage at 60ºC,while the value at the end of the 25th day was ranged from 6.25 to 7.6 mM O_2 /kg fat at 60 ºC. Similar results were also found by Archana, (2019) and report that ghee stored at 60 °C for 0 to 45 days had a peroxide value of 0 to 10.45 mM O_2/Kg. The result obtained are comparable with those of Narayanrao, (2007), who analyzed the ghee, S_{20} (Solid fraction at 20 ºC) and L_{20} (Liquid fraction at 20 ºC) sample contain 0 mM O_2/Kg at 0 day. As the storage day increased the peroxide value increase more in liquid fraction than solid fraction which containing more peroxide value than control sample. At 7 day of storage the ghee, S_{20} (Solid fraction at 20 ºC) and L_{20} (Liquid fraction at 20 ºC) sample contain 0.153, 1.4145 and 2.344 mM O_2/Kg fat.

The FFA value in ghee was increased with increasing levels of palm oil (5, 10 and 20 %) as similarly reported by Archana, (2019), who report that ghee stored at 29 °C for 0 to 60 day had FFA value of 0.33 to 0.60 (% OA). Niranjan, (2017), also reported that FFA content were ranged between 0.27 to 0.72 % OA in the market ghee samples against the value of 0.60 % OA recorded for control ghee sample at 0th day of storage. The rate of increase in FFA was very slow and gradual at the end of the 25th day of storage. The FFA content of control ghee was 0.80 % OA while the market samples were ranged between 0.57 to 0.96 % OA. The FFA content were ranged between 0.40 to 0.93 % OA in the desi ghee sample against the value of 0.60 % OA recorded for control ghee sample in the 0th day of storage. The rate of increase in FFA were very slow and gradual, at the end of the 25th day of storage the FFA content of control ghee were 0.80 % OA while the desi ghee samples ranged between 0.72 to 1.28 % OA for desi ghee Niranjan, (2017). Similar result were also found by Archana, (2019) and analysed that ghee stored at 29°C for 60 day had FFA content of 0.33 to 0.60 (% OA). Archana, (2019) also reported that ghee stored at 60 °C for 45 days had FFA content ranges from 0.42 to 0.61 (% OA). The shorter-chain homologues are principally responsible for the rancid flavour represented by butyric acid; FFA is undesirable in milk fat products (Munro et al. 1992). No work has been done on fractionated ghee in terms of the content of FFA.

Conclusion

It may be concluded from the present study, that as the palm oil level increased the antioxidant activity in the blended samples increased, this could be due to presence of higher levels of carotenoids and vitamins E in palm oil which act synergistically as powerful natural antioxidants and have also contributed towards stability against auto oxidation (peroxide value). The liquid fraction (L_{20} with palm oil 20%) has a higher antioxidant activity. Finally, the adoption of fractionation technique (especially liquid fraction) for the detection of adulteration with palm oil could be an effective method even at 5% level of adulteration. Still there is a scope for the detection at lesser concentration especially with DPPH method.

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Global Ghee Market: By Source: Cow, Buffalo, mixed; by end use: Retail, institutional; by distribution channel: supermarkets/hypermarkets, convenience stores, specialty stores, online, others; regional analysis; historical market and forecast (2018-2028); Market Dynamics: Competitive Landscape; Industry Events and Developments; https://www.expertmarketresearch.com/reports/ghee-market. [accessed 2022 November 19]

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