JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"STUDY OF PURE AND MARKETED GHEE SAMPLES"

Mayuri Shrikhande*[1], Dr. S.S. Khadabadi[2], Dr. S.L. Deore[2]

[1]Department of pharmacognosy, GCOP, Amravati
[2]Department of pharmacognosy, GCOP, Amravati

ABSTRACT

The Sanskrit word ghruta is the ancestor of the word ghee. Clarified butter fats are known as "ghee" in India. Making ghee may have existed long before the time of written records. The majority of milk products in India are made of ghee. Ghee production consumes around 28% of the milk produced in our nation. Regional preferences for ghee's flavor, colour, and texture differ within our nation. Most often, the desired qualities are obtained by carefully choosing the materials and controlling the manufacturing processes. The human diet must include ghee. It is the most significant component of meals. It has a high caloric value and is a great source of nutritional energy. Certain acids found in ghee are very important and necessary for humans. Ghee's supply is limited due to the market's high demand even though it serves as a vehicle for fat-soluble vitamins. The disparity between supply and demand leads to a number of errors and adulterations. The issue of adulteration has now assumed a very grave dimension. Print and electronic media have reported on the widespread practices of adulterating ghee, particularly in the country's centre and northern regions.

On sometimes, fresh and rancid ghee are combined together and sold in the market. Ghee samples that have been manipulated in any way vary in terms of their chemical and physical characteristics. In addition to getting advantage of people's money and messing with their religious beliefs, especially those of the vegetarian society, deceitful sellers are harming people's health by adding animal depot fats to ghee, the divine holy food.

KEYWORDS: Ghee, DPPH, Saponification value

INTRODUCTION

The word ghee is advanced from Sanskrit word ghruta. Ghee is a common Indian name for clarified butter fats. The origin of ghee making possibly lies a long way beyond recorded history.Ghee forms the largest segment of the milk products in India. Nearly 28 % of milk produced in our country is used for the production of ghee. The flavour, colour and texture preferences of ghee vary from region to region in our country. The desired properties are usually achieved through the way of selection of materials and manipulating the manufacturing conditions. Ghee, the pure clarified fat derived solely from milk or curd or from desi (cooking) butter orfrom cream with no added colorants or preservative. Ghee is composed of complex lipids of glycerides, free fatty acids, phospholipids, sterols, sterol esters, fat soluble vitamins, carbonyls, hydrocarbons and carotenoid. Ghee also contains traces of iron and calcium. The major component of ghee is glyceride which covers 98% of total material in ghee and rest 2% consistof sterols, commonly cholesterol occur to the extent of about 0.5 %. Ghee in its nature has low moisture content and antioxidant properties, it's sufficiently shelf stable. Several standards have been developed to regulate quality characteristics. According to standards, ghee should have minimum of 96% of fat, 0.3% maximum moisture, 0.3% maximum FFA (as oleic acid), peroxide value less than 1% Any kind of adulteration in ghee samples leads to the changes in the physical and chemical properties of ghee.

Ghee is a rich source of energy and vitamins A, D, E, and K. The essential fatty acids found in ghee cannot be synthesized in human body. Cow milk ghee is mostly preferred for Ayurvedic applications. Cow milk ghee is considered good for eyes, helps in digestion, nourishes body and gives strength. It increases appetite. The medicinal value of ghee also depends on the storagetime of ghee. Ayurvedic literature has reported about 50–60 types of medicated ghee. Plant extracts were selectively enriched in the preparation of medicated ghee. All fat-soluble therapeutic componentsof the herbs are retained in ghee. Medicated ghee has been used for various external and internal applications. Internal applications of medicated ghee include oral ingestion and an Ayurvedic treatment called panchakarma. Ghee is crucial a part of human diet. It is the most crucial element in meals. It is rich source of nutritional energy and includes excessive calorific value. The Ghee includes certain acids that are very crucial and essential for the human beings. They are vehicle for the fats soluble vitamins supply of ghee is less in marketplace for its excessive demand. The gap of availability and shortage results in several malpractices and adulteration. Ghee is an effective material in improving memory power, grasping power, and power to controlthe senses and to make them stronger. However, due to the shortage of ghee, and relatively greater demand, it is very expensive (costing 7 to 10 times more expensive than edible vegetable oils). Therefore, ghee is easily adulterated by the traders in the market. The commonly used adulterants are vegetable oils, animal body fats, mineral oils, starchy material, etc. The ghee is adulterated on a large scale in India. The most commonly used adulterants are vegetable oils and animal body fats. The detection of animal fats in ghee is difficult because the mixture has near about same physical and chemical characteristics. The adulterants like starch (Potato), sesame oil, dyes, artificial colours, vegetable fats, lard and waxes. Sometimes therancid ghee is also combined with the fresh ghee and is sold in the market. Any type ofadulteration in ghee

samples result in the changes in physical and chemical properties of ghee. By adding animal depot fats in ghee, the divine holy food, the unscrupulous traders are not only robbing the people's money, but also playing with the religious sentiments, especially of the vegetarian section of the society, besides adversely affecting their health. Recently, the problem of adulteration has taken a very serious dimension. Reports have been published in the print and electronic media, indicating the rampant malpractices of ghee adulteration, especially in the central and northern regions of the country.

EXPERIMENTAL WORK

Materials and instrumentsMaterials

Chemicals : Ethanol, Conc. Hydrochloric acid, Conc. Sulphuric acid Iodine solution, Potassium hydroxide, Sodium hydroxide, Nitric acid, Ammonia solution, Glacial acetic acid, Sodium carbonate, Ethyl acetate, Potassium iodide, Sodium thiosulphate, Glycerol, Amyl alcohol, Carbon disulphide, Furfural solution, Carbon tetrachloride, Ferric chloride, Potassium ferrocynide, Wij'sreagent, Acetic acid, Chloroform.

Procurement of pure cow ghee and pure buffalo ghee

The ghee was procured from the trusted ghee vendor in village Malakhed, Amravati. Cow and buffalo milks used for the preparation of respective ghee samples were obtained from local farmer maintaining a herd size of 10 animals. Cow milk was obtained from the herd of Jersey breed only and buffalo milk used was obtained from Murrah breed only. Cows and buffaloes were maintained under identical conditions of feeding and management. Cow and buffalo ghee were prepared by traditional creamery butter method under our observation and procured for the study.

Flowchart for the preparation of pure ghee samples was as follows:

Collection of Cow/ Buffalo milk

↓

Warming milk to 40^{0}C

↓

Separation of cream

↓

Churning cream to butter

↓

Heat clarification at 120^{0}C

↓

Filtration using Muslin clothGhee

Procured pure cow and buffalo ghee

Sensory evaluation

All the market samples of ghee were evaluated for their sensory characteristics. The sampleswere evaluated for their appearance and colour, body flavor and texture and overall acceptance.

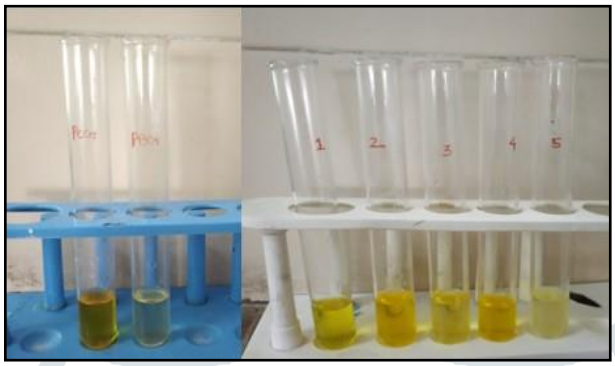


Fig. 1 Sensory evaluation

Melted ghee samplesDetermination of physico-chemical characteristics

Melting point

The temperature at which previously solidified fat turns completely into liquid state known as melting point. If the melting occurs within a specific temperature range then it is calledthe melting interval.

Fig. 2 Melting point apparatus

Melting point apparatus-Detection of presence of added colours

Despite the ban, yellow colours such as annatto, turmeric and coal tar dyes are sometimesadded to buffalo ghee so that it can pass off as cow ghee, which fetches a higher price.

About 5g of ghee sample was added to the 10 ml of solvent mixture of carbon disulphide and alcohol (2:15) in a separating funnel and the contents were shaken. The partition of colour tothe upper alcohol layer indicated the presence of added colours such as annatto, turmeric, ceres yellow 39, ceres orange and Sudan 1. Carotene and browning pigments remain with the fat.

Development of kit for the detection of adulteration in gheeDetection of starch in ghee:

Starch can be determined by using iodine solution. (Khandelwal) The reaction mixture shows blue-deep blue colour if the starch is present in the ghee sample. If there is no colour change to blue colour, it indicates that starch is not admixed in the ghee.

Detection of added colour

Yellow colours such as annatto, turmeric and coal tar dyes are sometimes added tobuffalo ghee so as to sell it as cow ghee, which fetches a higher price in the market. In order to detect the presence of added colour in the ghee following procedure was carried out.

Sensory evaluation of ghee samples

The ghee samples were subjected to sensory evaluation for appearance and colour,flavour, body and texture and overall acceptance. The sensory evaluation for the pure ghee samples and marketed ghee samples are presented in Table 1. The cow ghee samples werehaving light to deep yellow colour whereas buffalo ghee sample were having creamy yellowishto white colour.

An observation of the findings revealed that the flavor of pure ghee samples were more pleasant than those for the marketed ghee samples under the study.

Most of the ghee samples exhibited granular texture with fine to coarse grains. One sample (No.5) was found to have waxy texture (Table 6.1). In general, it was observed that the pure ghee samples showed better sensory evaluation, when compared to the marketed ghee samples.

Table: 1 Sensory evaluation of ghee samples

Sample code	**PCG**	**PBG**	**01**	**02**	**03**	**04**	**05**
Appearance and colour	Deep yellow colour	Whitecolour	Yellow Colour	Deep yellow colour	Light yellowish colour	Deep yellow colour	Creamy yellowish colour
Flavour (smell and taste)	Creamy butter like with nutty flavour	Creamy butter like	Creamy butter like with nutty flavour	Creamy butter like with nutty flavour	Creamy butter with nutty flavour	Creamy butter like with nutty flavour	Not so pleasant flavour
Body and texture	Granular texture with coarse grains	Granular texture	Granular texture with fine grains	Granular texture with coarse grains	Granular texture	Granular texture with coarse grains	Waxy texture

The grainy texture of ghee is reported to be influenced by the fatty acid composition (Joshi and Vyas, 1976), and the fatty acid composition is influenced by seasonal factors (Rama Murthy and Narayanan, 1971). The varying granular texture of marketed ghee samples observed in the present study could be due to variation in the fatty acid composition.

Physico-chemical properties of ghee samples

Fats and oils exhibit certain physical and chemical properties, which have been employed for their characterization and differentiation since long time The Bureau of Indian Standards published the best known methods for the determination of these characteristics, which are accepted as standard procedures in India ISI, 1981.

The determined analytical properties are the reflections of the nature of the constituents, which make up the fat. Fats are almost entirely triglycerides of fatty acids. Since glycerol only constitutes about 10 %, it is the nature of the fatty acids and the manner of distribution of these acids as glycerol triesters, which determine the various characteristicsexhibited by the whole fat.

Saponification value:

In the present study, the saponification values of the pure ghee samples were in the range of 225.0 to 229.0. The saponification values of the marketed ghee samples were in the range of 199.15 to 234.2.

Iodine value:

The iodine value for pure ghee samples ranged from 32.7 to 36.37. Iodine value is a measure of the extent of unsaturation of ghee. It was reported to be between 26 and 41 for ghee (Laksminarayana and Rama Murthy, 1985). Iodine value of ghee samples from the market were in the range of 29.89 to 51.0. The sample No. 4 was found to have iodine value of 51.0. This showed that this sample was possibly adulterated with some vegetable oils.

Reichert-Meissl value

RM value is considered to be an important characteristic to detect adulteration of ghee with foreign oils/fats. The average value for ghee (milk fat) samples reported was

22.6 – 34.5 (Laksminarayana and Rama Murthy, 1985). Of the ghee samples analysed from the market, the RM value ranged from 1.1 to 33.22 showing wide variation. The sample No.4 failed to meet the standards, showed extremely low RM values of 1.1, this clearly indicates the gross adulteration this sample was subjected to.

Polenske value:

Polenske value is one of the specifications of AG Mark. The data of polenske value of samples of pure ghee is shown in Table 3. It showed that PV of ghee samples ranged from 1.3 to

1.5 which was within the limits of AG marks. Thus all the samples pass the test for AG Mark standards (1 to 2). Polenske value of the marketed ghee samples ranged from 0.4 to 2.0. Of the 5 samples analysed, one sample (No.4) showed value of 0.4. The low RM and Polenske values clearly indicate that the sample No.5 was grossly

adulterated.

Melting range:

In the present study the melting range of pure ghee samples varying from 23°-38°C. For the marketed ghee samples the range was found to be 24° - 39°C. All the ghee samples showed the melting point within the range.

Moisture content:

The moisture content of the pure ghee samples was in the range of 0.05 to 0.21 %. A similar trend was exhibited by the marketed ghee samples. Thus all the samples met the standards for moisture by PFA (max. 0.5 %) as well as AG Mark (Max. 0.3%). Earlier, Sharma and Zariwala (1978) reported moisture content of 0.02 to 0.7 % for market ghee samples.

Table: 2 Physico-chemical characteristics of ghee samples

Sample code	**PCG**	**PBG**	**01**	**02**	**03**	**04**	**05**
Saponification value	225.8	228.6	232.8	221.5	234.2	199.15	227.2
Iodinevalue	36.3	32.7	33.5	29.89	36.8	51.0	36.9
Reichert-Meissl value	29.15	32.45	27.72	27.06	33.22	1.1	26.51
Polenske value	1.3	1.5	1.8	1.3	2.0	0.4	1.6
Melting range(°C)	24-38	23-37	25-37	26-38	24-37	25-38	26-38
Moisture(%)	0.05	0.06	0.15	0.11	0.18	0.13	0.21

Free fatty acids:

In the present study, the pure ghee samples were found to have the free fatty acids (FFA) in the range of 0.16 to 0.95% in terms of oleic acid. The marketed ghee samples were found having the FFA in the range of 0.27 to 1.3 % as oleic acid. The above results showed that the FFA contents of all the samples were within the limit prescribed by the PFA and the AG Mark standards. These results are in the agreement with the findings of other workers (Rao *et al.*,2004).

Small proportions of fatty acid are always present in fresh milk fat. Small amounts of free fatty acids in ghee (~ 0.15 % oleic) did not affect its keeping quality, but high acid ghee deteriorated faster. (Lalitha and Dastur, 1953)

Peroxide value

The peroxide values in the pure ghee samples and marketed ghee samples were found to be in the range of 0.2 to 0.3mM of O_2/kg and 0.7 to0.9 mM of O_2/kg respectively. It was seen from the data that none of the sample had shown the development of peroxides. The age of the samples at the time of the analysis was in the range of 1 to 4 months.

Table: 3 free fatty acids, peroxide value of ghee samples

Sample code	PCG	PBG	01	02	03	04	05
FFA (% oleic acid)	0.16	0.95	0.27	1.3	1.0	1.17	1.22
Peroxide value (mM of O_2/kg fat)	0.3	0.2	0.7	0.8	0.9	0.7	0.9

Detection of adulteration in ghee samples

Ghee is an expensive product, costing three times as much as edible vegetable oils. Thus its adulteration is a very profitable proposition and one widely practiced. Once ghee has been isolated, a wide variety of materials are employed for adulteration. Various vegetable oils viz. coconut oil, palm oil, etc. are in common use. Tallow or other animal body fats being hard and firm cannot be detected visually in ghee even when present in considerable proportion. In fact blends of tallow and vegetable oil can be prepared which look remarkably like ghee. Mineral oilsor solid paraffin are considerably cheaper than ghee and can be used as adulterants. The hydrogenated fats are also used to adulterate ghee. Despite the ban, yellow colours are sometimes added to buffalo ghee so as to pass it of as cow ghee, which fetches a higher price.Detection of added colours:

According to the PFA (2004) Rules, the ghee should be free from added colours. When the ghee samples from the market were tested to detect the presence of added colours,none of the ghee sample was found to have added colours.

Table: 4 Detection of added colour

Sample code	Observation	Inference
PCG	 No colour Partition	No added colour
PBG	 No colour Partition	No added colour

Detection of mineral oil: (Holde's Test)

Unlike oils and fats, mineral oils are not saponifiable by alkali. On this basis there was a test to detect the presence of mineral oil in ghee. None of the ghee sample showed turbidity hence, tested negative with respect to mineral oil.

Detection of vanaspati (Baudouin test):

In the present study, the ghee samples from the market were tested for the presence of vanaspati. Out of the 5 marketed ghee samples 4 samples tested negative while 1 sample (No. 4) showed positive Baudouin test.

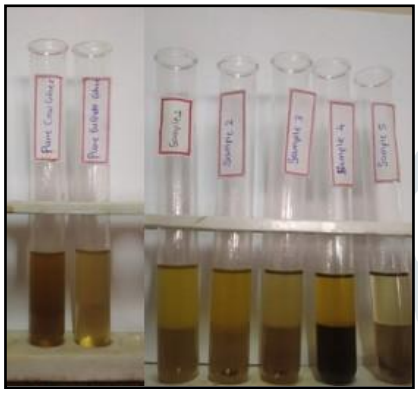


Fig. 1 Baudouin test for Ghee samples

Table: 5. Presence of added colours, mineral oil and vanaspati in ghee samples

Sample code	PCG	PBG	01	02	03	04	05
Added colours	-	-	-	-	-	-	-
Mineral oil	-	-	-	-	-	-	-
Vanaspati	-	-	-	-	-	+	-

Detection of presence of animal body fat in gheeCrystallization time test

The ghee samples were subjected to crystallization test. Results of the present study revealed thatcow ghee took ~ 16 to 20 min for crystallization which is almost similar to that of buffalo ghee. On the contrary, 1 marketed ghee samples out of 5 crystallized very fast taking only few secondsto 13 min (Table 6.6). One sample (No. 4) did not show any crystallization even after 30 min ofincubation at 17°C. This indicated that the samples could be suspected for adulteration. Based onthe physicochemical characteristics, it was found that the samples No. 4 had low RM, Polenskeand saponification values and high Iodine values indicating a possible adulteration with vegetable oils. The rest of the samples exhibited a normal range of these characteristics. However, the crystallization time of one sample (No.5) was so less that the sample could be suspected for adulteration with animal body fats.

Table: 6 Crystallization time (min) for samples

Sample code	Degree of crystallization			
	Negative	Slight	Fair	Excellent
	Time (min)			
PGC	15.5	16	17	19
PBG	15	16	18	19
S1	15.5	16	17	19
S2	16.5	17	18	19.5
S3	14.5	15.5	17	18
S4	18.5	20	24	-
S5	4.5	5.0	6.0	8.0

Fractionation of ghee

In the present study, ghee samples from market were subjected to fractionation at 25°Cfor 12 h and the yield of liquid and solid fractions were measured. The pure ghee, after fractionation, found to have about 15 % liquid fraction and 85 % of solid fraction. Table 9 shows the fractionation behaviour of the ghee samples. The yield of liquid and solid fractions ofmarketed ghee samples showed a similar range; liquid fractions ranged from 9 – 18 % and solid fractions ranged from 82 – 91 % except, sample No. 5 showed a very less liquid fraction and one sample (No.4) showed a very high proportion of liquid fraction (Table 9). These observations matched with those observed for crystallization behaviour of the same samples, confirming the possible adulteration.

Table: 7. Yield of fractions of ghee samples obtained at 25°C

Sample code	PCG	PBG	S1	S2	S3	S4	S5
% SolidPart	84	82	88	86	91	11	96
%LiquidPart	16	18	12	14	09	89	04

Methods based on specific constituentsHalphen's test:

A characteristics crimson colour was not developed by any of the test samples, which indicates that none of the marketed ghee sample contain cotton seed oil and thus tested negative for the Halphen's test.

Detection of presence of palm oil in ghee:

When 2 ml of pure ghee and marketed ghee samples were allowed to react with the said chromogenic solution; it was observed that green colour persisted in pure ghee beyond 2 minutes after the addition of chromogenic solution; similarly marketed ghee samples showed persistant green colour, thus no colour turned from green to

deep blue colour (purssian blue) after 2 minute of the addition of the chromogenic solution. This indicates that none of the sample was adulterated with palm oil.

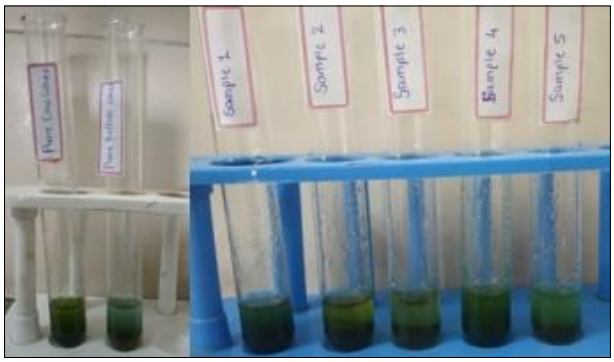


Fig.2 Detection of palm oil in Ghee samples

Antioxidant activity of ghee samples

To evaluate the free radical scavenging activity of the test samples, the ghee sampleswere diluted to 10ug, 20ug, 30ug, 40µg and 50µg per ml with ethyl acetate solution and 1ml of DPPH solution was added .After 30min the absorbance is measured at 517nm.the percentage of the DPPH radical scavenging is calculated by equation; % Radical scavenging activity = x 100

Table: 8 Observation of Antioxidant activity of standard (Ascorbic acid)

Concentration(µg/ml)	Absorbance	Control	%RSA
10	0.048	0.43	88.83
20	0.036	0.43	91.62
30	0.027	0.43	93.72
40	0.019	0.43	95.58
50	0.012	0.43	97.20

Fig.3 Calibration curve of standard

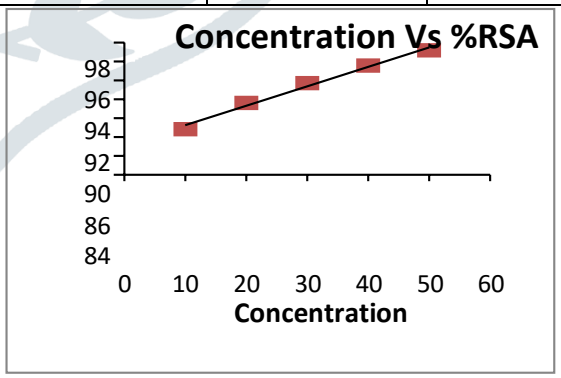


Fig.4 Antioxidant activity of standard

Table: 9 Observation of Antioxidant activity of PBG

Concentration (µg/ml)	Absorbance		Control	%RSA
10	0.082		0.43	80.93
20	0.072		0.43	83.25
30	0.063		0.43	85.34
40	0.054		0.43	87.44
50	0.042	0.43	90.23	

Table: 10 Observationof Antioxidant activity of S1(Amul cow ghee

Concentration(µg/ml)	Absorbance	Control	%RSA
10	0.095	0.43	77.90
20	0.085	0.43	80.23
30	0.079	0.43	81.62
40	0.072	0.43	83.25
50	0.064	0.43	85.11

Fig. 5 Calibration curve of PBG

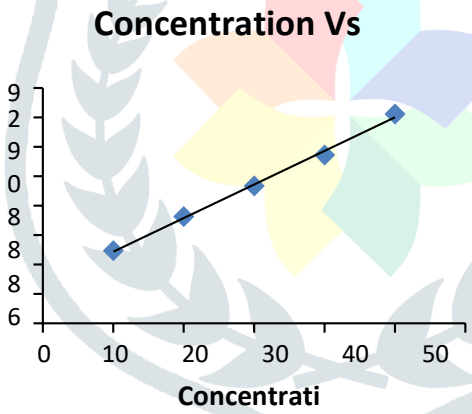
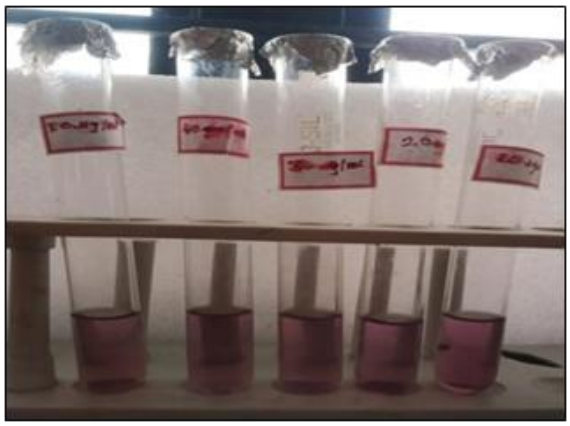


Fig.6 Antioxidant activity of PBG



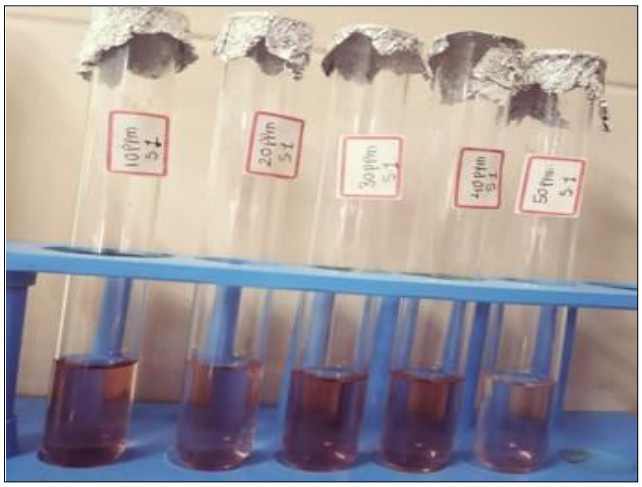


Fig.7 Calibration curve of S1

Table: 11 Observation of antioxidant activity of S2 (Patanjali cow ghee)

Concentration(µg/ml)	Absorbance	Control	%RSA
10	0.099	0.43	76.97
20	0.084	0.43	80.46
30	0.067	0.43	84.41
40	0.052	0.43	87.90
50	0.042	0.43	90.23

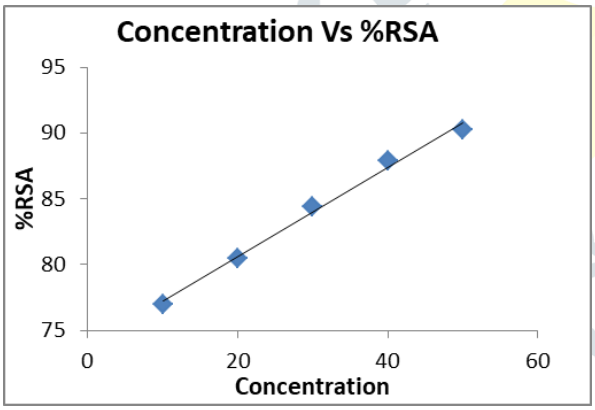


Fig.8 Calibration curve of S2

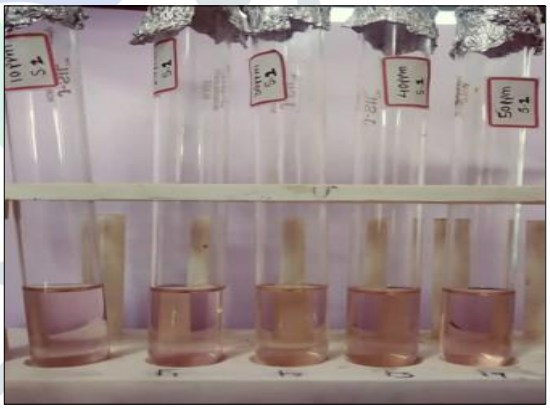


Fig.9 Antioxidant activity of S2

7.5.6 Sample 3 (Anik buffalo ghee)

Table: 12 Observation of Antioxidant activity of S3 (Anik buffalo ghee)

Concentration (µg/ml)	Absorbance	Control	%RSA
10	0.11	0.43	74.41
20	0.104	0.43	75.34
30	0.097	0.43	77.44
40	0.088	0.43	79.53

50	0.082	0.43	80.93

Fig.10 Calibration curve of S3

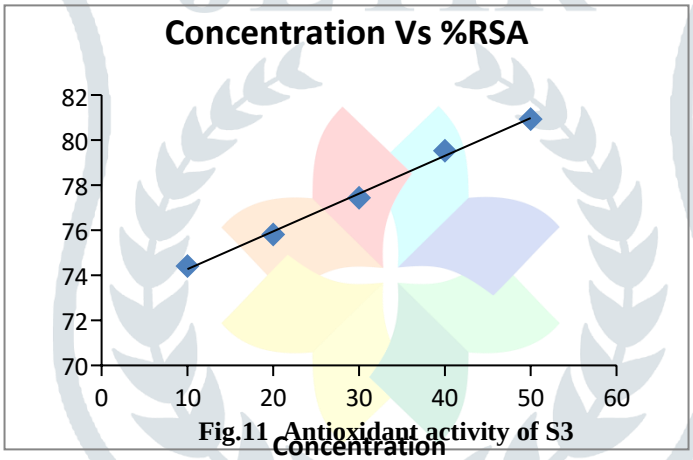


Fig.11 Antioxidant activity of S3

Sample 4 (Gowardhan cow ghee

Table: 13 Observation of Antioxidant activity of S4 (Gowardhan cow ghee)

Concentration (µg/ml)	Absorbance	Control	%RSA
10	0.098	0.43	77.20
20	0.087	0.43	79.76
30	0.075	0.43	82.55
40	0.066	0.43	84.65
50	0.052	0.43	87.90

Fig.12 Calibration curve of S4

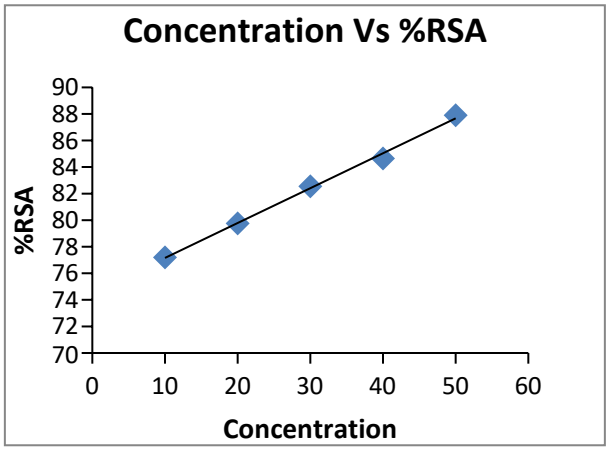
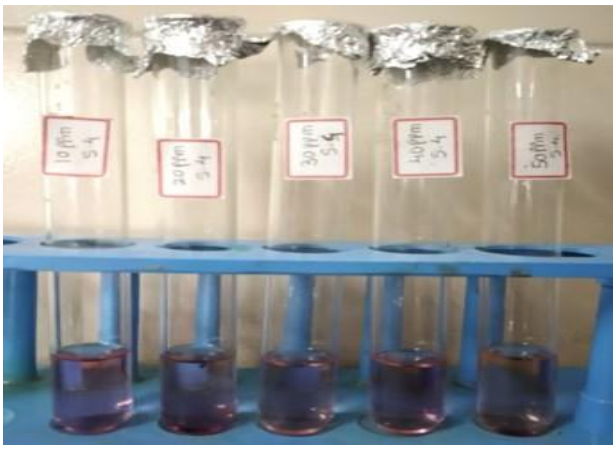


Fig.13 Antioxidant activity of S4



Sample 5 (Dinshaw buffalo ghee)

Table: 14 Observation of Antioxidant activity of S5 (Dinshaw buffalo ghee)

Concentration(µg/ml)	Absorbance	Control	%RSA
10	0.143	0.43	66.74
20	0.126	0.43	70.69
30	0.108	0.43	74.88
40	0.097	0.43	77.44
50	0.085	0.43	80.23

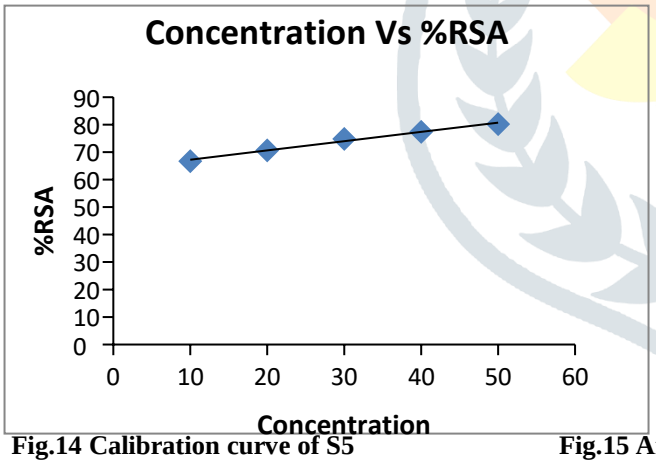


Fig.14 Calibration curve of S5

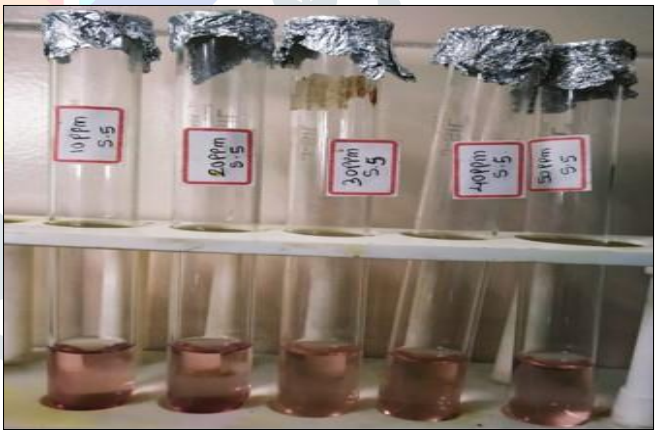


Fig.15 Antioxidant activity of S5

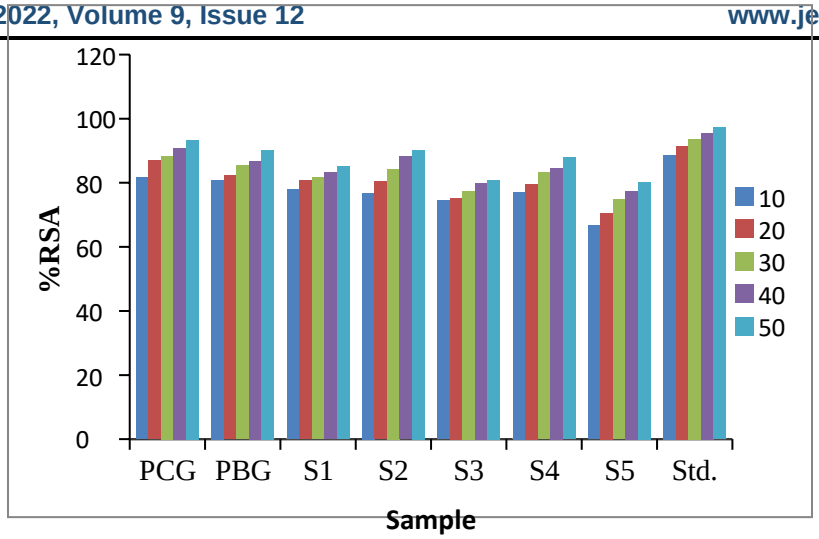


Fig.16 Graph of Antioxidant activity of ghee samples with standard

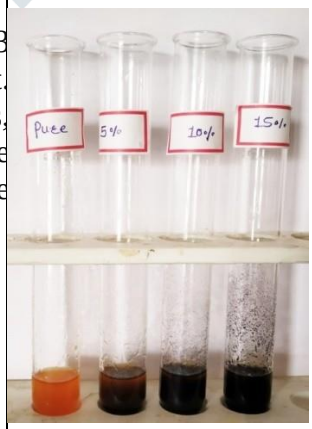
In evaluating the antioxidant activity of the ghee samples, the DPPH (2,2-diphenyl-1- picryl-hydrazyl-hydrate) free radical technique was used. The assay is based on the measurement

of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. This free radical, steady at room temperature, reduces in the existence of an antioxidant and it give rise to the reduced form with the loss of violet colour.

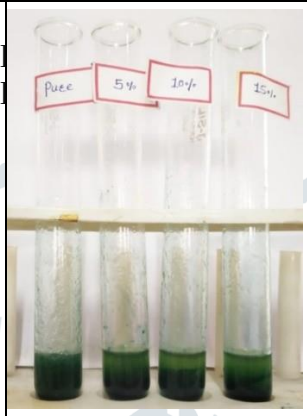
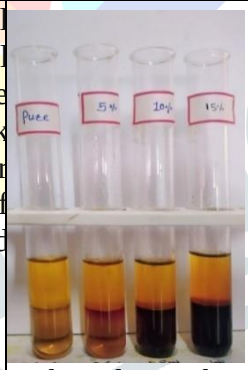
The antioxidant activity was inspected in PCG, PBG and 5 marketed ghee samples. The excessive antioxidant activity was shown by PCG (93.48 %) was compared with the S1 (85.11%), S2 (90.23%) and S4 (87.90%) cow ghee and PBG (90.23%) which was compared with S3 (80.93%) and S5 (80.23%) buffalo ghee, against the standard (Ascorbic acid) which was found to be 97.20%. From this study it was found that cow ghee shows more antioxidant activity than buffalo ghee.

Development of kit for the detection of adulteration

Table: 15 Tests for kit development

Sr. No.	Test	Procedure	Observation	Inference
1	Iodine test	2ml ghee sample+ 2-3 drops of Iodine reagent. If blue colour appears, it indicates the presence of starch in the ghee.	 Blue colour was observed in	Intensity of blue colour increases as the conc. of starch adulterant increases. So this test can be used to detect the presence of starch inghee.

			the adulterated test samples	
2	Test for added colour	10ml solvent mixture of carbon disluphide and alcohol + 5ml test sample + shake well. The partition of yellow colour in the Upper alcohol indicates the presence	Yellow colour was partitioned in the upper	Yellow colour partitioned in the upper alcohol layer which indicates that the presence of added colour.

		of added colour	 layer	
3	Test for Palm oil	2ml test sample + 1ml ferric chloride + 0.3ml potassium ferricynide + shake well and observe	 Pure ghee showed green colour while adulterated samples showed purssin blue colour.	As the conc. of palmoil
	Test for vanaspati	5ml test sample + 5ml Conc. HCl + 0.4ml Furfural solution. The appearance of pink colour in the lower layer indicates the presence of sesame oil or hardened vegetable oil (vanaspati).	 pink colour observed at the junction of two liquid	The intensity of pink colour increases as the Conc. of adulterant increases.

REFERENCES

i. Ahmed, S., Hamid, M.and Rahman, M. (2020). Assessment of ghee adulterated with oils and fats in Bangladesh. *Journal of advanced veterinary and animal research*, Vol 7, No. 4, page 678–684.

ii. Annonymous (2015). Manual of Methods of Analysis of Foods, Food Safety and Standards Authority of India, Ministry of Health and Family Welfare, Govt. of India, New Delhi.

iii. Ayari, F., Ghaleh, E.M., Rabbani, H. and Heidarbeigi, K. (2018). Detection of the adulteration in pure cow ghee by electronic nose method (case study: sunflower oil and cow body fat). *International Journal of Food Properties,* ISSN: 1094-2912.

b. Bailey, A.E. Harris, J.H. and Skau, E.L. (1966). Cyclopropenoid fatty acid content and fatty acid composition of crude oils from twenty-five varieties of cottonseed. *J. Am. Oil Chem. Soc.,* 43: 107-110.

i. Becon, S. and Bector, B. S. (1998). Status of unsaponifiable constituentsof milk fat of Karan Swiss and Karan Fries crossbred cows, *Indian J.Dairy Sci.*, 51: 61-62.

ii. Bharwade, M., Balakrishnan S. and Chaudhary N. (2018). Chemical Constituents and Autoxidative Stability of Milk Lipids of Kankrej Cow. *International Journal of Current Microbiology and Applied Sciences,* Volume 7, No. 03, ISSN: 2319-7706.

c. Bhat, G. S., Murthy, M. K. R. and Rao, M. B. (1980). Autooxidation of milk fat 1.Development of peroxides and carbonyl compounds during storage of cow and buffalo milk fats. *Milchwissenschaft-Milk Sci. Int.,* 35: 284-286.

d. Bindal, M.P. and Jain, M.K. (1973) A note on the unsaponifiable matter of ghee and its quantitative relationship with cholesterol content, Indian J. Anim. Sci., 43:900-902

2. BIS: 3508(1966) Methods of Sampling and Test for Ghee (Butter fat), Bureau of Indian Standards, Manak Bhavan, New Delhi.

3. Derewiaka, D., Sosin´ska, E., Obiedzin´ski, M., Krogulec, A. and Czaplicki, S. (2011). Determination of the adulteration of butter. *Eur. J. Lipid Sci. Technol.,* 113, 1005–1011.

4. Dhurvey, Y.R., Kawtikwar, P.S. and Sakarkar, D.M. (2012). Evaluation of physic- chemical properties of cow ghee before and after hydrogenation. *International Journal ofChemTech Research*, 4(1): 185-189.

5. Gandhi, K. and Lal, D. (2017). Butyro-Refractometer (B.R.) reading linked with solvent fractionation technique as an aid to detect adulteration of palm olein and sheep body fatin ghee. *Indian Journal of Natural Products and Resources,* Vol. 8(3), pp. 276-281.

6. Gandhi, K., Upadhyay, N., Aghav, A.D., Sharma, V. and Lal, D. (2014). Detection of adulteration of ghee (clarified milk fat) with palmolein and sheep body fat using Reichert-Meissl (RM) value coupled with solvent fractionation technique. *Indian J. DairySci*., 67(5).

7. Hazra T., Sharma V., Sharma R. and Arora S. (2017). A species specific simplex polymerase chain reaction-based approach for detection of goat tallow in heat clarified milk fat (ghee). International journal of food properties, Vol 20, No. S1, S69-S75.

8. Hazra, T., Sharma, V., Saha, P. and Pratapsinh, P.M. (2017). Physico-chemical properties analysis based approaches to ascertain the purity of ghee-a mini review. *International Journal of Science, Environment and Technology*, Vol. 6, No 1, 2017, 899 – 907.

9. Jariwala, K.N. (2014). Analytical techniques for the assessment of Physico-chemical properties of ghee. *Indian Journal of Applied Research*, (4) 6: 216-217.

10. Joshi, C.H. and Vyas, S.H. (1976) Studies on buffalo ghee: II various conditions affecting the Granulation of ghee, Indian J.Dairy Sci., **29:** 13 – 17.

11. Khandelwal K. R. Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan, pg-21.

12. Kumar, A., Goyal, S.K., Munesh, V.K. and Kumar, L. (2016). Study on physico- chemical analysis of ghee. *South Asian J. Food Technol. Environ.,* 2(3&4):448-451.

13. Kumar, A., Kumar, A., Lal, D., Seth, R. and Sharma, V. (2010). Validation of ultra-violet and visible

spectroscopic methods for detection of milk fat adulteration. *J. Dairying,foods & H. S.*, Vol. 29, No. 1, 29 (1) : 8 – 14.

14. Kumar, A., Lal, D., Seth, R and Rajan, S. (2002) Recent trends in Detection of Adulteration in Milk Fat A Review, *Indian J. Dairy Sci.*, 55:319 330.

15. Kumar, A., Upadhyay, N., Padghan, P.V., Gandhi, K., Lal, D. and Sharma, V. (2015). Detection of Vegetable Oil and Animal Depot Fat Adulteration in Anhydrous Milk Fat

(Ghee) using Fatty Acid Composition. *MOJ Food Processing & Technology,* Volume 1Issue 3, 1(3): 00013

16. Laksminarayanan, M. and Rama Murthy, M.K. (1985) Cow and Buffalo milk fatfractions: Part

17. Lalitha, K.R. and Dastur, N.N. (1953) Keeping quality of ghee, Indian J. Dairy Sci., **6**:147-156.

18. Narayanan, K.M., Anantakrishnan, C. P. and Sen, K.C. (1956). Co-Vitamin studies. Part

I. Variations in tocopherol and vitamin A contents in milk and butterfat of cows andbuffaloes. *Indian J. Dairy Sci.*, 9: 44-51.

19. Panda, D. and Bindal, M.P. (1998). Detection of adulteration in ghee with animal body fats and vegetable oils using crystallization test. *Indian Dairyman.* 50(9):13-16.

20. Pandey, A. and Jariwala, K.N., (2015). Detection of adulteration in ghee from markets of Ahmedabad by FTIR spectroscopy. *Journal of Chemical and Pharmaceutical Research*, 7(6):10-14.

21. Pandya, N.C. and Kanawjia, S.K., (2002). Ghee: A traditional nutraceutical. *IndianDairyman*, 54, 67–75.

22. Pathania, P., Sharma, V., Arora S. and Rao, P.S. (2019). A novel approach to detect highly manipulated fat adulterant as Reichert–Meissl value-adjuster in ghee (clarified butter) through signature peaks by gas chromatography of triglycerides. *J. Food Sci. Technol.*

23. Pena-Serna, C. and Restrepo-Betancur, L.F. (2020). Chemical, physicochemical, microbiological and sensory characterization of cow and buffalo ghee. *Food Science and Technology*, 40(Suppl. 2): 444-450.

24. Rama Murthy, M.K. and Narayanan, K.M. (1971) Fatty acid composition of buffalo and cow milk fats by GLC. *Milchwissenshaft*, 26: 693 – 697.

25. Rama Murthy, M.K. and Narayanan, K.M. (1972). Polyunsaturated fatty acids of cowand buffalo milk fat. *Milchwissenshaft*, 27: 695 – 698.

26. Ramani, A., Hazra, T., Parmar, M.P., Sindhav, R.G. and Ramani, V.M. (2019). A simple rapid technique for detection of palm oil in ghee. *Indian J Dairy Sci*, 72(4): 441-444.

27. Ramya, S.B., Baskaran, D., Vijayarani, K., Palanidorai, R. and Ramasamy, D. (2016). A Study on Physico-Chemical Properties of Uthukuli Ghee. *International Journal of Current Microbiology and Applied Sciences*, Volume 8, No. 04 ISSN: 2319-7706.

28. Rao, L.V.; Rangadham, M. and Rao, B.V.R. (2004) Quality of milk products marketed in Hyderabad city Part II-Chemical quality of milk products, Indian J. Dairy Sci., **57:** 171- 176.

29. Ray, P.R. (2019). Technological and Biochemical Aspects of Ghee (Butter Oil). *Engineering Practices For Milk Products*, chapter 5:84 -107.

30. Roy, S.C. and Sen, A.N. (1961) Detection of added colour in ghee and butter, J. Proc. Instn. Chem.

(India), 33:13-15.

31. Sharma, R.S. (1981). Ghee: A Resume of Recent Researches, *J. of Food Sci. and Tech.,* 18: 70-77.

32. Sharma, U.P. and Zariwala, I.T. (1978) Survey of quality of milk products in Bombay, *J. Food Sci. Tech.,* 15:118-121.

33. Sindhuja, S. , Prakruthi, M., Manasa, R. , Shekhara Naik, R. and Shivananjappa M. (2020). Health benefits of ghee (clarified butter) - A review from ayurvedic perspective. *IP Journal of Nutrition, Metabolism and Health Science*, 3(3): 64-72.

34. Singhal, O.P. (1980). Adulterants and methods for detection. *Indian Dairyman*. 32: 771- 774.

35. SP:18 (1981) Handbook of Food Analysis. Part XI Dairy Products. Manak Bhavan, New Delhi: Bureau of Indian Standards.

36. Venkatachalam, V. and Sundaram, S. (1957). Test for mineral oil in edible oils and ghee.*Current Sci.,* 26(5): 157.

37. Wadhwa, B .K. and Jain, M. K. (1990). Chemistry of ghee flavour – A review, *Indian J.Dairy Sci*., 43: 601