

# Effect of Fractionation on the Composition, Physico-Chemical Properties, and Oxidative Stability Index of HF Ghee

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## Abstract

*Ghee, the clarified milkfat prepared mainly from cow or buffalo milk and has the highest share of exports in the dairy product sector is increasingly demanding and now it is gaining popularity in the western world too. Modification of milk fat composition in the simplest way of fractionation would result in fat fractions with desired technical and nutritional properties, appears which to be the most promising option. The increase/decrease in milk fat parameters and their impacts by milk fat fractionation technologies have been studied by optimizing the fractionation temperature to 300°C. The composition and physicochemical properties of all the ghee fractions were pertaining to the Ghee standards making it to be possible to be marketed in either way., stearin or olein, depending on the consumer ' s choice. Economic fractionation of milk fat into liquid and solid fat fractions, which differ markedly from one another in chemical composition and physical characteristics, but are within the statutory limits of food safety by FSSAI could increase the utilization of milk fat in many food applications. The apparent merits of fractionated milk fat in the baking and confectionery sector have encouraged several scientists and efforts were made to incorporate fractions for the preparations of sweets. The potential of having a solid fraction for increased textural properties and overall acceptability was identified in many products and such initiatives will help to come up with the concept of tailoring milk fat to compete with other fats and oils in meeting the needs of the food industry.*

**Keywords:** Fractionation, HF Ghee, Physico-Chemical Properties, Oxidative Stability Index,

## Introduction

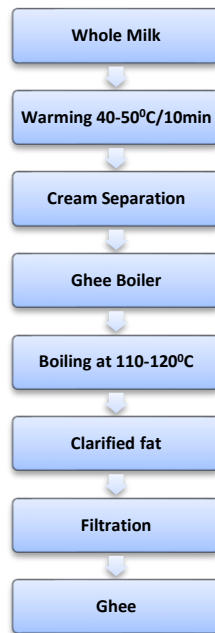
India ranks first in milk production in the world, producing 187.7 million tons (BAHS) whereas the world's milk production amounts to 827.88MT (FAOSTAT 2019). India contributes to around 22.67%, of global milk production. Besides fluid milk consumption, the consumption of value-added dairy products is also growing due to higher incomes, urbanization, and demographic changes. Ghee is the most consumed value-added dairy product and represents the second-largest dairy product in India, after liquid milk. The demand for ghee (clarified butter) and butter is robust. At present, ghee is witnessing an appreciable growth in demand across India, and the ghee market is to reach a value of INR 4,653 billion by 2024 because of an increase in disposable incomes, which has permitted all level consumers to afford it. Ghee does not require any special mode of preservation strategies and the product itself is shelf-stable and is exclusively preferred by all groups of people. The adulteration of Ghee, especially with refined and hydrogenated vegetable oils, is the major hindering factor that obstructs market growth in India. The ghee market showed a slight deceleration among affluent classes as fear of fat is stalking Urban India (Sinha and Tiwari, 2007). Ghee has widespread popularity in India and in many other countries such as Sudan, Ethiopia, and the Middle East (Kumar *et al.*, 2000). The last decade had witnessed an increase in the production of cow ghee between 3000 and 12000 tons per year in the American continent (FAO, 2019). This may be attributed to the globalization process and the replacement of the consumption of margarine due to the high content of industrial trans fatty acids (iTFA). Ghee has a characteristic flavour which is the basic criterion for its acceptance and is greatly influenced by the processing methods i. e., fermentation of cream, butter, or milk, and even heating processes. The relatively lower moisture content and possible natural antioxidants make it shelf-stable. The characteristic short-chain fatty acids have a major role in making the product responsible for its better digestibility and anti-cancer properties. Ghee is also an important carrier of fat-soluble vitamins (A, D, E, K) and essential fatty acids (linolenic acid and arachidonic acid), apart from having short-chain fatty acids. Ghee possesses many properties and is believed to be a coolant, capable of increasing mental power, physical appearance, and prognosis of ulcers and eye diseases. The belief that dietary saturated fatty acids (SFA) should be reduced to minimize the metabolic syndrome and cardiovascular disease (CVD) risk has dominated nutritional guidelines for decades and the high content of saturated fatty acids in milk fat (about two-thirds of total fatty acids) has been many times used as an argument to link dairy product consumption with an increased incidence of those pathologies. Natural fats have an intricate composition as they are composed of several fatty acids present primarily as triacylglycerols. They provide milk fat with highly diverse functional and nutritional properties. This complexity of fat provides opportunities for modifying themselves for different applications. The diversified fat group of having both long-chain, short-chain, saturated, and unsaturated fatty acids mixture has initiated the thought of having different technologies to produce modified fat with different physicochemical or nutritional properties. Modified fats have a great scope of application. They can modify the conventional stereotypical fats and can also be used as ingredients in functional foods and also as nutraceuticals. It is interesting to note that one can use different fractions derived from a single source of fat for different applications. The modification of fat can be considered a tool that enhances the functionality and nutritional value of natural fats and broadens their area of application (Gandhi *et al.*, 2013).

## Materials and Methods

The experiments were carried out in the Department of Dairy Chemistry, Dairy Science College, Hebbal, Bengaluru. The pooled milk samples of Holstein Friesian were collected from the University Livestock Farm, Hebbal, Bengaluru. Ghee samples were prepared using the Direct creamery method with a clarification temperature of 120°C in a batch process and stored in airtight glass bottles. These samples were used for the purpose of analysis. All the chemicals and reagents used were of 'AR' grade unless otherwise specified;

### ***Preparation of Ghee (Sserunji et al., 1998)***

The process of Ghee preparation is provided in the schematic diagram.



### ***Fractionation of Ghee***

The Ghee was fractionated using the technique, Fractionation processing (Deffense, 2000). The ghee was melted at 60 °C to remove the crystal memory. It was allowed to slowly cool to 30 °C and held at this temperature for 24 h in an incubator for crystallization. The liquid was separated from the crystals by decantation after centrifugation at 2000 rpm for 10 min in a temperature-controlled centrifuge (REMI) maintained at 30°C. The solid fraction obtained at 30 °C (S<sub>30</sub>) was coined as the High Melting Fraction (HMF or S<sub>30</sub>). The weight of the solid fraction thus obtained was determined and the percent yield was calculated. Subsequently, the liquid portion obtained at 30 °C was termed to be Low Melting Fraction (LMF or L<sub>30</sub>).

### ***Determination of Moisture Content***

The moisture content of ghee samples was determined by the methods as described in FSSAI Manual, Oils and Fats, 2015. The details of the procedure are as follows:

**Procedure:** About 10 g of the ghee sample was weighed into a moisture dish that had been dried previously, cooled in desiccators, and then weighed. The dish was then placed in the hot air oven for approximately one hour at 105±1°C. The dish was then removed from the oven, cooled in a desiccator to room temperature, and weighed. The procedure of heating at 105°C for 30 min, cooling, and weighing was repeated until the difference between the two successive weighing did not exceed 1 mg. The moisture content was calculated by the following formula and the different fat content in the ghee samples was calculated.

$$\text{Moisture content (\% by weight)} = \frac{100 \times W_1 - W_2}{W_1 - W_2}$$

Where,

W<sub>1</sub>=Weight in g of the dish with ghee before drying,  
 W<sub>2</sub>= Weight in g of the dish with ghee after drying, and  
 W= Weight in g of the empty dish

### ***Estimation of Free fatty Acid Content***

Free fatty acid (FFA) levels, expressed as % oleic acid, of ghee samples, were determined by the method IS: 548 (Part 1) - 2010, which is described below:

**Procedure:** Ten grams of ghee sample was accurately weighed in a 250 ml conical flask. 50 ml of neutralized alcohol (at 70°C) was added into the flask containing ghee. The contents were brought to boil on a boiling water

bath. The solution, while hot, was titrated against 0.1N sodium hydroxide solution, shaking vigorously during titration. The end point of the titration was perceived when the addition of a single drop produced a slight but definite colour change (pink colour) for at least 15 s. The FFA levels were expressed as per percent oleic acid.

$$\text{FFA (\% oleic acid)} = \frac{2.82 \times V}{W}$$

Where,

V-volume (ml) of NaOH required for titration of a sample

W-weight of the sample

$W_w$  = weight of the bottle with water

$W_e$  = empty weight of the bottle

### **Estimation of Cholesterol Content**

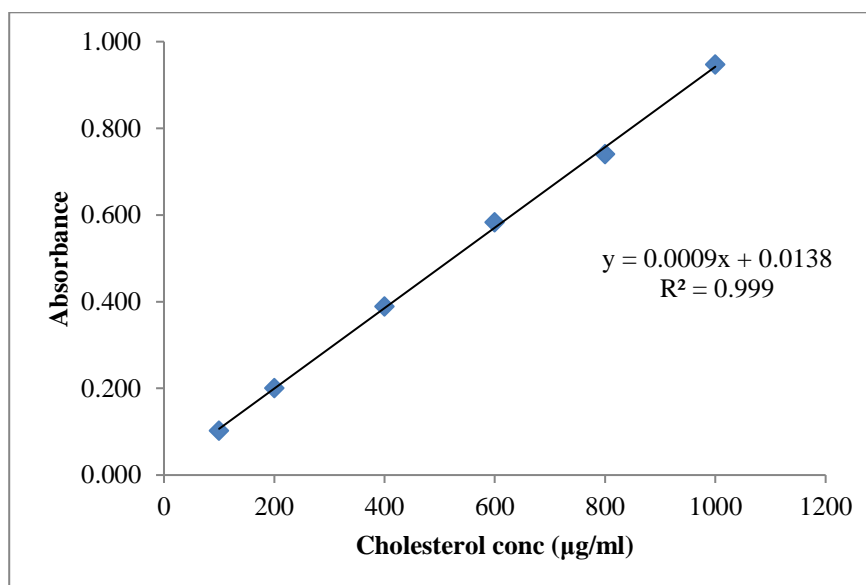
The cholesterol content of ghee was estimated as per the method given by Sharma *et al.*, (2007).

**Procedure:** Exactly 5 gm of molten, clear anhydrous milk fat was taken in a 25 ml volumetric flask. Dissolved in chloroform and made the volume to 25 ml with chloroform. Pipetted out 2 ml of this above solution in a glass stoppered test tube. To this added 1 ml of chloroform and 4 ml of freshly prepared cold LB reagent. Incubated the tubes at 25°C for 10 min in a water bath to develop the colour. Absorbance was recorded in a spectrophotometer at 650 nm.

### **Preparation of Standard Curve**

**Preparation of cholesterol stock solution:** Taken 0.5 gm of cholesterol standard, dissolved it in chloroform, and transferred the contents to a 100ml volumetric flask, and made the volume to 100ml with chloroform.

**Preparation of cholesterol working solution:** Exactly 2 ml of the stock solution was taken in a 25 ml volumetric flask. Made the volume to 25 ml with chloroform. Aliquots of 0.0, 0.50, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of cholesterol working solution were taken in glass stoppered test tubes. Made the volume to 3.0 ml by adding the required amount of chloroform in respective tubes. Added 4 ml of LB - reagent in all the tubes and kept them in a water bath maintained at 25°C for 10 min. Recorded the absorbance at 650 nm. Plotted absorbance readings against the corresponding cholesterol amount (~g) to get a standard curve. Amount of cholesterol present in the sample was calculated from the standard curve.



### **Estimation of SFA, USFA, MUFA, PUFA & Trans fat**

The Saturated fat (SFA), Unsaturated fat (USFA), Mono Unsaturated fat (MUFA), Poly Unsaturated fat (PUFA), and Trans-fat percent are determined by the fatty acid profile which is done by gas-liquid chromatography (GC) and

the details are detailed in FSSAI Manual, Oils and Fats, 2015.

### ***Estimation of Fatty Acid profile***

The fatty acids were estimated as per the methods in FSSAI Manual, Oils and Fats, 2015. Analysis of fatty acids from biological or food samples generally involves the following steps: Extraction of lipids, conversion of the extracted lipids to fatty acid methyl esters (FAME), and analysis of the FAME using gas-liquid chromatography (GC) for the fatty acid profile.

Procedure: FAME was prepared by taking the 0.5 g ghee collected from all the HF, S<sub>30</sub>, and HF L<sub>30</sub> fat. To these samples 1 mL internal standard was added (Supelco, 37 Component FAME Mix 1 ml having the varied concentration in dichloromethane). The concentration of the sample was made to 0.5 mg/mL in methanol by dilution, to this mixture 0.7 mL of 10 N KOH was added with 5.3 mL methanol and then incubated for 30 min at 55°C with 15 min. the interval of shaking. The mixture was cooled, and 0.58 mL of 24N H<sub>2</sub>SO<sub>4</sub> was added and again incubated as mentioned above. Then the mixture was cooled and 3 ml hexane was added followed by vortexing for 5 min. and then the contents were centrifuged at 3000 rpm for 5 min. The upper solvent layer was collected and transferred to glass ampoules and sealed and then stored at -20°C till the end of the analysis. Then these samples were subjected for GC analysis (Model: 7890A GC (Agilent) using an FID detector as per the below-mentioned parameters. Tridecanoic acid was used as an external standard in the analysis.

**Column specifications:** Type: SP<sup>TM</sup>-2560 Capillary Column; Column No: 62694-04; Dimensions: 100m x 0.25mm x 0.2µm film thickness; Make: Supelco Sigma-Aldrich.

**Experimental conditions:** Inlet temperature: 250°C; Injection volume: 1µL; Split ratio: 50:1; Carrier gas: Hydrogen, 18cm/sec at 175°C; Detector: FID, 280°C.

### ***Determination of Size and Quantity of Grains***

The size and quantity of grains formed during granulation of ghee were determined as per the procedure given by the Manual of Fats and Oils by FSSAI,2015.

### ***Sensory evaluation of Ghee***

The sensory evaluation of ghee samples from HF and its fractions was done using the ISI scorecard. A trained sensory panel assessed the coded ghee samples at random, according to the ISI scorecard. Sensory evaluation of the ghee samples was carried out with a twelve-member panel (ages 22 to 50 years) who were Scientists, Students, and technical staff of the Institute with previous knowledge of sensory evaluation of dairy products. Samples were placed in airtight glass bottles and conditioned at room temperature before testing. Samples were analyzed and the respective panel's scores were recorded.

### ***Storage Stability Test***

The Rancimat apparatus was used to measure the induction periods of ghee samples, to access the storage stability.

#### **Operating Parameters**

1. Instrument temperature: 120°C
2. Air flow rate: 20 l/h
3. Sample size: 3 g

**Procedure:** Prior to the use of Rancimat, it was imperative that all the glassware was thoroughly cleaned as per the operating instructions in the Rancimat manual. A 3.0 g sample of completely melted ghee was weighed accurately into each of the reaction vessels. The vessels were then placed in the heating block of the Rancimat apparatus. Vessels were then connected to the measuring vessels via connecting tube. Sixty milliliters of de-ionized water were measured into each of the measuring vessels, containing the electrodes. The measuring vessels were also placed in the Rancimat apparatus. All the parts were connected to the apparatus as per the operating instructions, and the test was carried out until the endpoints all the samples were reached.

## Results & Discussion

### Moisture

The moisture content of solid and liquid fractions of ghee samples prepared from HF Ghee was subjected to moisture analysis and there was no significant difference among the fractions. The moisture content is within the limit of 0.5(max) set by FSSAI for all fractions. The unique keeping quality of Ghee is due to its low moisture content. The moisture content of ghee prescribes to be 0.3 and it should not be more than 0.5% as per AGMARK and FSSAI standards.

### Free Fatty Acids

The free fatty acid content of ghee samples prepared from the HF ghee and their solid and liquid fractions were successively 0.45, 0.39, and 0.47 for whole HF Ghee, S<sub>30</sub>, and L<sub>30</sub> fractions, respectively. The values were found low in S<sub>30</sub> fractions compared to L<sub>30</sub> fractions however there was no significant difference observed between the values(P<0.05). There was no significant difference between the fractions of the HF sample. Free fatty acid in fat promotes microbial growth and decreases the shelf life of fat. The FFA content of ghee depends upon the method of manufacture of ghee, the initial quality of cream or butter used for making ghee, heat clarifying temperature, and the residual moisture content. The FFA content was within the limits of reference standards such as FSSAI and AGMARK.

### Cholesterol Content of Ghee Fractions from Different Sources

The cholesterol content was higher in whole HF ghee when compared to its S<sub>30</sub> and L<sub>30</sub> counterparts. The cholesterol content was 290.50, 287.67, and 285.70 for HF, S<sub>30</sub>, and L<sub>30</sub> ghee samples (Table 1). There was a significant difference between the cholesterol content of S<sub>30</sub> and L<sub>30</sub> ghee to the whole HF ghee whereas the S<sub>30</sub> and L<sub>30</sub> had shown no significant difference(P<0.05). The reason for the lower level of cholesterol in solid and liquid fractions compared to whole ghee would have been attributed to the dissociation of cholesterol esters into both fractions at 30 c when the dry fractionation method was used. The results for cholesterol in solid fraction were in Tandem with those of Lopez *et al.*, 2006, and Arul *et al.*,1998.

**Table 1:** Effect of Fractionation of milk fat on the composition of HF ghee

Source of ghee	Moisture (%)	FFA (%Oleic acid)	Cholesterol (mg/100g fat)	SFA (%)	USFA (%)	MUFA (%)	PUFA (%)	Trans Fat (%)
HF ghee	0.18 <sup>a</sup>	0.45 <sup>a</sup>	290.50 <sup>a</sup>	66.36 <sup>a</sup>	33.64 <sup>a</sup>	31.21 <sup>a</sup>	2.52 <sup>a</sup>	1.03 <sup>a</sup>
HF S <sub>30</sub>	0.16 <sup>a</sup>	0.39 <sup>a</sup>	287.67 <sup>ac</sup>	68.80 <sup>a</sup>	31.20 <sup>a</sup>	28.45 <sup>b</sup>	2.12 <sup>a</sup>	1.11 <sup>a</sup>
HF L <sub>30</sub>	0.17 <sup>a</sup>	0.47 <sup>a</sup>	285.70 <sup>c</sup>	65.43 <sup>b</sup>	34.57 <sup>b</sup>	32.39 <sup>a</sup>	2.96 <sup>a</sup>	0.98 <sup>a</sup>
CD(P≤0.05)	0.06	0.12	3.31	2.68	2.96	2.12	1.00	0.38

Similar superscripts indicate non-significant (NS) at the corresponding critical difference (CD); HF = Holstein Friesian; S<sub>30</sub> = Solid fraction at 30<sup>o</sup> C; L<sub>30</sub> = Liquid fraction at 30<sup>o</sup> C

### Saturated Fatty Acid

The percentage of saturated fatty acid in HF whole ghee, S<sub>30</sub>, and L<sub>30</sub> were 66.36,68.80 and 65.43(Table 1). The percent of saturated fatty acid was more in S<sub>30</sub> fraction but the difference was not significant (P<0.05). The results were within the range of the findings of the fatty acid profile of Carolina *et al.* , 2018 and Helena *et al.*, 2008.

### Unsaturated Fatty Acid

The percent of unsaturated fatty acids was 33.64,31.20 and 34.57 (Table 1) for whole HF ghee, S<sub>30</sub>, and L<sub>30</sub> fractions, respectively. The trend observed was that the unsaturated fatty acid content was significantly higher in all the liquid fractions whereas it was lower in solid fractions. The authors Carolina *et al.*, 2018, Helena *et al.*, 2008, and Małkowska *et al.*, 2021 have also reported similar results.

### **Monounsaturated Fatty Acid (MUFA)**

The percent of MUFA in whole HF Ghee, S<sub>30</sub>, and L<sub>30</sub> fractions, respectively was 31.21, 28.45, and 32.39 (Table 1). The L<sub>30</sub> fraction showed a significant increase (P<0.05) in the percent of MUFA. The percentage of Monosaturated fatty acid MUFA was present in greater proportion in the L<sub>30</sub> fraction For HF Ghee (32.39%) as compared to the S<sub>30</sub> fraction (28.45).

### **Polyunsaturated Fatty Acid (PUFA)**

The PUFA percent was more in the L<sub>30</sub> fraction. The values were 2.52, 2.12, and 2.96 (Table 1) whole HF Ghee, S<sub>30</sub>, and L<sub>30</sub> fractions. The results are in agreement with the findings of Antony *et al.*, 2018, Małkowska *et al.*, 2021 and Manickavasagan *et al.*, 2014.

### **Trans Fat**

The trans-fat percent was estimated to be 1.03, 1.11, 0.98 (Table 1) for whole HF Ghee, S<sub>30</sub>, and L<sub>30</sub> fractions. The difference was not significant in the percent of trans-fat amongst the HF ghee fractions. Fatty acids that contain conjugated trans double bonds are considered separate entities and can be called Natural Trans fats such as conjugated linoleic acid (Indu *et al.*, 2021) and it was present in HF Ghee.

### **Fatty acid Profile in HF Ghee and its Fractions**

It was evident from the table-2 that there was only a marginal difference between the content of fatty acids among the fatty acid profile of whole Ghee samples as well as their S<sub>30</sub> and L<sub>30</sub> counterparts. The short-chain fatty acids are concentrated more in the L<sub>30</sub> fraction. S<sub>30</sub> was enriched in long-chain saturated fatty acids (LCSFA) (C16:0 and C18:0) and reduced in short-chain fatty acids (SCFA) (C4:0–C8:0), medium-chain fatty acids (C10:0–C14:0) and long-chain unsaturated fatty acids (LCUSFA). The myristic, stearic, and palmitic acids were concentrated in the S<sub>30</sub> fraction. This was found true for all three varieties of ghee samples Conversely, LMF were depleted in LCSFA, while SCFA and LCUSFA dominated their composition. The results did not show significant differences (Po0:05) between S<sub>30</sub> and L<sub>30</sub>, which clearly indicates their identical physical characteristics. These findings are in agreement with the results obtained by Fatouh *et al.*, 2003 and Grall and Hartel 1992.

**Table 2:** Effect of fractionation on the Fatty Acid Profile of HF Ghee

Fatty acid	HF		
	WMF	S <sub>30</sub>	L <sub>30</sub>
C 4:0 Butyric acid	1.01	1.09	1.15
C 6:0 Caproic acid	1.36	1.31	1.46
C 8:0 Caprylic acid	1.07	0.99	1.11
C 10:0 Capric acid	2.32	2.17	2.36
C 12:0 Lauric acid	2.71	2.73	2.05
C 14:0 Myristic acid	9.66	9.69	9.45
C 15:0 Pentadecanoic acid	0.93	0.92	0.89
C16:0 Palmitic acid	28.41	29.05	27.37
C 16:1 Palmitoleic acid	1.23	1.17	1.26
C 17:0 margaric acid	0.79	0.80	0.77
C 18:0 Stearic acid	14.24	15.19	14.11
C 18 :01 Oleic acid	28.06	25.74	28.59
C 18:1n9t Elaidic acid	0.45	0.48	0.35
C18:1 n11t Vaccenic acid	0.10	0.10	0.10
C18:2 (Linoleic acid) cis-9,12	1.83	1.61	1.84
C18:2 (Linoleladic) trans-9,12	0.34	0.39	0.35
C18:3 (Linolenic acid)	0.20	0.19	0.22
Conjugated Linoleic acid	0.10	0.10	0.10
C20:0 Arachidic acid	1.04	1.14	0.98

All values are average of three trials; HF = Holstein Friesian; WMF= Whole milk fat

### Sensory evaluation of ghee of different fractions

Ghee from different sources was fractionated and subjected to a panel of judges by providing the ISI scorecard to evaluate with respect to attributes., flavour, texture, colour, free from impurities, and packaging. The scores recorded for the attributes are detailed in Table 3.

**Table 3:** Effect of fractionation on the Physico-chemical attributes of HF Ghee

Physico-chemical constants	HF ghee			
	WMF	S <sub>30</sub>	L <sub>30</sub>	CD
RM Value	27.56 <sup>a</sup>	26.63 <sup>a</sup>	28.76 <sup>a</sup>	2.20
Polenske value	1.77 <sup>a</sup>	1.67 <sup>a</sup>	1.84 <sup>a</sup>	0.54
Iodine value	33.10 <sup>a</sup>	29.40 <sup>b</sup>	33.90 <sup>a</sup>	1.10
Saponification Value	224.6 <sup>a</sup>	227.1 <sup>b</sup>	223.10 <sup>c</sup>	1.40
Melting Point	34.00 <sup>a</sup>	36.5 <sup>b</sup>	31.50 <sup>c</sup>	1.89
BR Reading	41.72 <sup>a</sup>	41.70 <sup>a</sup>	41.73 <sup>a</sup>	0.86
Size of fat globule	0.220 <sup>a</sup>	0.250 <sup>b</sup>	Waxy	0.25

Note: All values are average of three trials, Similar superscripts indicate non-significant (NS) at the corresponding critical difference (CD); HF = Holstein Friesian; WMF=Whole milkfat; S<sub>30</sub> = Solid fraction at 300 C; L<sub>30</sub> = Liquid fraction at 300 C

- Flavour:** The whole ghee scored the highest among the three varieties of ghee samples. There was a significant difference in the flavor score of whole HF Ghee and its S<sub>30</sub>, and L<sub>30</sub> fractions. The flavor score of HF L<sub>30</sub> Ghee was the highest followed by whole ghee and S<sub>30</sub> and the flavor score was significantly different (P<0.05) from each other.
- Texture:** The score secured (out of 20) for texture attributes for HF, S<sub>30</sub>, and L<sub>30</sub>., was 17.50,17.04, and 16.01. The score was significantly different (P<0.05) between the whole ghee, S<sub>30</sub>, and L<sub>30</sub> fractions.
- Colour:** The whole ghee of HF, secured the highest score owing to its superiority in colour compared to its S<sub>30</sub> and L<sub>30</sub> counterparts however there was no significant difference between the whole ghee and S<sub>30</sub> Ghee. The colour score of L<sub>30</sub> Ghee was significantly(P<0.05) lower in Ghee prepared from different sources of milk fat.
- Free from impurities:** The score for Whole Ghee and Its Fractions remained the same for the three varieties of Ghee and there was no significant difference (P<0.05) amongst the three fractions of ghee samples.
- Packaging:** The packaging score for Whole Ghee and Its fractions remained the same for the three varieties of ghee and there was no significant difference(P<0.05) observed among the three kinds of ghee samples.
- Overall Acceptability:** The overall acceptability score was the highest for the whole fraction and was significantly different (P<0.05) from the L<sub>30</sub> and S<sub>30</sub> fractions (Table 4). The score of Whole Ghee, S<sub>30</sub>, L<sub>30</sub> was 93.59, 91.89, and 91.62, respectively. The data on sensory evaluation of S<sub>30</sub> and L<sub>30</sub> fractions as such were not available however the values were within the range of sensory score of ghee by various researchers (Luis *et al.* , 2020 , and Abdel *et al.*, 2013).

**Table 4:** Effect of fractionation on the sensory attributes of HF Ghee

Source	Sensory attributes					Overall Acceptability (100)
	Flavor (50)	Texture (20)	Color (10)	Free from impurities (15)	Packaging (5)	
	Average Scored allotted by the panel of judges					
HF	45.50 <sup>a</sup>	17.50 <sup>a</sup>	8.82 <sup>a</sup>	14	5	93.59 <sup>a</sup>
HF S <sub>30</sub>	44.20 <sup>b</sup>	17.04 <sup>b</sup>	8.52 <sup>a</sup>	14	5	91.89 <sup>b</sup>
HF L <sub>30</sub>	46.45 <sup>c</sup>	16.01 <sup>c</sup>	7.59 <sup>b</sup>	14	5	91.62 <sup>b</sup>
CD						
(P≤0.05)	0.85	0.41	0.43	NS	NS	0.44

Note: Sensory evaluation using ISI score card; All values are average of three trials; Similar superscripts indicate non-significant at the corresponding critical difference (CD); HF = Holstein Friesian; S<sub>30</sub> = Solid fraction at 30<sup>o</sup> C; L<sub>30</sub> = Liquid fraction at 30<sup>o</sup> C

## g) **Effect of fractionation on Physico-chemical constants of HF ghee**

### i. **Reichert Meissl Value (RM) value**

The RM value is a measure of steam volatile water-soluble fatty acids (Butyric and caproic acid). It was determined for different ghee samples. The values for WMF, S<sub>30</sub>, and L<sub>30</sub> were 27.56, 26.63 and 28.76., and 26.75 for whole HF, S<sub>30</sub>, and L<sub>30</sub> Ghee respectively. The RM values were not significantly different between the fractions of HF Ghee however it was found higher in L<sub>30</sub> (P<0.05).

### ii. **3.11.2 Polenske value**

The Polenske value is the measure of steam volatile water-insoluble fatty acids (Caprylic and capric acid) and it was determined for different variants of HF ghee samples. The values were 1.77, 1.67, 1.84 whole HF, S<sub>30</sub>, and L<sub>30</sub> Ghee, respectively. The values were not significantly different from each other (P<0.05).

### iii. **3.11.3 Iodine value**

The iodine value for WMF, S<sub>30</sub>, and L<sub>30</sub> was 33.10, 29.40, and 33.90 for whole HF, S<sub>30</sub>, and L<sub>30</sub> Ghee, respectively attributing to the higher number of unsaturated fatty acids in L<sub>30</sub>. The values recorded were higher for low melting fractions of HF Ghee but not significantly different from that of whole Ghee and S 30 Ghee.

### iv. **3.11.4 Saponification value**

Saponification value denotes the number of milligrams of KOH required to saponify one gram of fat and which gives an indication of the average molecular weight of fatty acids present. The saponification value for WMF, S<sub>30</sub>, and L<sub>30</sub> were 224.60, 227.10, 223.10, whole HF, S<sub>30</sub>, and L<sub>30</sub> Ghee, respectively. The S<sub>30</sub> fraction of each variety of Ghee showed significantly higher values (P<0.05) whereas those of the L<sub>30</sub> fraction recorded lower values in all three fractions.

### v. **3.11.5 Melting point**

The melting point was determined for different ghee samples. The values were 34, 36.5 and 31.5, for whole HF, S<sub>30</sub>, and L<sub>30</sub> Ghee, respectively. The highest value recorded was for S<sub>30</sub> fraction of HF Ghee attributing to more number of saturated fatty acids in the solid fraction.

### vi. **3.11.6 Butyro Refractometer Reading (BR Reading)**

The BR readings recorded were 41.72, 41.70 and 41.73., 41 for whole HF, S<sub>30</sub>, and L<sub>30</sub> Ghee, respectively and there was no significant difference (P<0.05) observed among all ghee samples.

### vii. **Size of fat globule**

The size of crystallization of milk fat after fractionation is studied and the values recorded were 0.220, 0.250 whole HF, S<sub>30</sub>, and Ghee, respectively. The crystals were waxy in Liquid ghee samples (L<sub>30</sub>) and could not be measured.

## h) **Oxidative Stability of Ghee and its Fractions from Different Sources**

Rancimat was used to determine the time of induction of the ghee samples (Table 4). Among the tested ones, the longest induction time (the highest oxidative stability) recorded was for S<sub>30</sub> ghee sample followed by the Whole ghee and L<sub>30</sub> ghee samples. The values were 18.93, 19.50, 7.60 hr for HF whole ghee, S<sub>30</sub> ghee and L<sub>30</sub> fraction. There was a significant difference between L<sub>30</sub> to S<sub>30</sub> and whole ghee (P< 0.05).

Among the tested ones, the longest induction time (the highest oxidative stability) recorded was for the S<sub>30</sub> ghee sample followed by the Whole ghee and L<sub>30</sub> ghee samples. The oxidative stability would have been linked to the unsaturated fatty acids and their presence makes the fat less stable. The oxidative stability of the analyzed oils probably also depended on the different content of pro- and antioxidant compounds (Magdalena *et al.*, 2018). However, estimations from these methods do require lot many data points at different temperatures and the

correlation database needs to be developed due to the widely divergent kinetics of lipid oxidation. Therefore, an accurate prediction of the shelf-life of edible fats and oils in normal storage conditions by an accelerated shelf-life test is still a sector to be studied more by the scientific community.

**Table 5:** Effect of fractionation on the Oxidative stability of Hf Ghee

Source of ghee	Whole	S <sub>30</sub>	L <sub>30</sub>	CD
Holstein Friesian	18.93 <sup>a</sup>	19.50 <sup>a</sup>	7.60 <sup>b</sup>	2.82

*Note:* All values are average of three trials; Similar superscripts indicate non-significant (NS) at corresponding critical difference (CD); S<sub>30</sub> = Solid fraction at 30<sup>o</sup> C; L<sub>30</sub> = Liquid fraction at 30<sup>o</sup> C; Oxidative Stability in Hours

## Conclusion

The effect of fractionation was studied on the composition of ghee and it was revealed that there was no significant difference in moisture or FFA amongst the fractions. The levels are within the range specified by FSSAI. The cholesterol content was higher in whole ghee when compared to its S<sub>30</sub> and L<sub>30</sub> counterparts among all the milk fats. The decreasing order of cholesterol content was whole milk fat, >S<sub>30</sub>>L<sub>30</sub> ghee samples. The L<sub>30</sub> fraction had recorded the least cholesterol content in the ghee samples. The L<sub>30</sub> Ghee had shown a lesser content of saturated fatty acid among all the samples. The unsaturated fatty acid content was significantly higher in all the liquid fractions whereas it was lower in the solid fraction. The order of decreasing trend for unsaturated fat was L<sub>30</sub> >WMF> S<sub>30</sub>. The fatty acid profile upon fractionation was studied and it was found that there was only a marginal difference between the content of fatty acids among the fatty acid profile of the whole Ghee sample as well as their S<sub>30</sub> and L<sub>30</sub> counterparts. The short-chain fatty acids are concentrated more in the L<sub>30</sub> fraction. S<sub>30</sub> was enriched in long-chain saturated fatty acids (LCSFA) (C16:0 and C18:0) and reduced in short-chain fatty acids (SCFA) (C4:0–C8:0), medium-chain fatty acids (C10:0–C14:0) and long-chain unsaturated fatty acids (LCUSFA). The myristic, stearic, and palmitic acids were concentrated in the S<sub>30</sub> fraction. The RM value, Polenske value, and Iodine Value were found higher in the L<sub>30</sub> fraction the S<sub>30</sub> fraction whereas Melting Point and Saponification value were found higher in the S<sub>30</sub> fraction for all the samples of Ghee. The BR reading has shown no significant difference among the three fractions. The sensory evaluation has proved that the whole ghee has got higher overall acceptability however the S<sub>30</sub> and the L<sub>30</sub> fractions were also acceptable but at a significantly lower level when compared to whole ghee. The effect of fractionation on oxidative stability of different ghee samples and their fractions were studied and it was proved that the longest induction time (the highest oxidative stability) recorded was for the S<sub>30</sub> ghee sample followed by whole ghee and L<sub>30</sub> ghee samples. The oxidative stability would have been linked to the unsaturated fatty acids and their presence makes the fat less stable.

## Contribution by Authors

Equal contribution

## Conflict of Interests

There is no conflict of interest.

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