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# Fractionation as a tool to detect adulteration in ghee: a Review

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**ABSTRACT**
Fractionation is a temperature-controlled technique of separating triacylglycerol based on their melting temperatures. The fractionation technique was implemented to provide a distinct separation of addition adulterants into separate fractions, particularly solid and liquid milk fat fractions. The ghee samples were separated so that the majority of the animal body fat was concentrated mostly in solid fraction and the vegetable oil concentrated in the liquid fraction.

**Keywords:**Fractionation, Adulterated ghee, Technique detection, Solid fraction, Liquid fraction

## I. INTRODUCTION

Milk and milk products are essential components of the human diet, especially for vulnerable populations like pregnant women,lactating mother'schildren and elders. It provides energy and nutrition for the human body nourishment. The conversion of liquid milk into high-value food products is more profitable than the sale of liquid milk itself (Aneja et al. 2015). Ghee is one such value-added product of Indian origin. In the Indian subcontinent, ghee is among the most popular traditional milk products (Duhan et al. 2018). As per the Food Safety and Standards Authority of India (FSSAI) regulation (2017), Milk fat, ghee, butter oil, anhydrous milk fat and anhydrous butter oil are fatty products derived exclusively from milk or products obtained from milk or both using processes which result in almost total removal of water and milk solids-not-fat. Ghee has especially developed flavour and physical structure as a result of its method of manufacturing.

Ghee is a clarified butterfat derived from cream or butter and is a popular dairy product in India because of its pleasant flavour and semisolid (granular) texture. Ghee is mainly composed of triacylglycerol complex lipids (98%), along with free fatty acids, phospholipids, sterols, hydrocarbons, carbonyl compounds and fat soluble vitamins (A, D, E, and K), and carotenoids in case of cow ghee.

Ghee is superior to other fats because of its distinct flavour and pleasant aroma, andunique fatty acid profile especially short- and medium chain fatty acids, oleic acid and conjugated linoleic acid. As a result, ghee plays a significant role in human nutrition. The short-chain fatty acids of gheehave been reported to promote digestion. It can encourage the growth of bifidobacteria, a good bacterium found in the intestine that has been associated with a lower risk of colon cancer(Gosewade et al. 2017). Ghee is known for boosting cognition and healing a variety of physiological ailments such as ulcers. Conjugated linoleic acid (CLA), sphingomylein, butyric acid, and myristic acid present in ghee have been shown to accord anticarcinogenic property. Ghee has been utilised in the manufacture of various Ayurvedic and Unani medications since ancient times in India. Ghee It has been utilised in Ayurvedic treatment. In India, ghee is used for frying and cooking as well as for garnishing, dressing, and spreading on a variety of foods.

Indian consumers consider granularity in ghee as a significant requirement for quality and purity. Coarse grains are evenly dispersed throughout the liquid component of an excellent granular ghee. The textural quality of ghee has an impact on the texture of various food preparations in which it is used. Ghee is a very expensive product that is about three to fourtimes the price of edible vegetable oil (Gosewade et al. 2017).

The adulteration of ghee has harmed the dairy industry's image both in India and abroad. The severity of the problem can be evaluated by the numerous incidents of adulteration reported in the press. Detecting adulterants was much more difficult when changes in the composition of milk fat were made, which is affected by the season, species, and diet fed to the animal.

The composition varies with the adulteration of milk fat with vegetable oil, especially in triacylglycerol and sterol content. Because of the varied composition of the triglycerides contained in milk fat, detecting adulterants has always been difficult. The measurement of physical-chemical characteristics,

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elements of unsaponifiable matter, and evaluation of water-soluble and insoluble volatile fatty acids have all been used to detect foreign fats in milk fat. Animal body fat (sheep fat) and vegetable oil (palm oil) are inexpensive, so they are suspected to be used as adulterants in milk fat (Gandhi et al.2014). To detect adulteration in milk and milk products with foreign fats, thin layer chromatography (TLC) of whole and unsaponifiable matter of milk fat, gas chromatography (GC) analysis of triacylglycerol (TAG) or fatty acid profile, and HPLC analysis of TAG and marker sterols of milk fat in combination with multivariate statistical data processing have been used. On the other hand, the majority of the above-mentioned characteristics are only effective when large amounts of adulterants are employed, and they are not capable of detecting the type and level of added adulterants.

One of the most cost-effective methods of altering the physical properties of milk fat is fractionation. Fat is separated into fractions depending on melting temperature, solidification temperature, and volatility of triacylglycerol, as well as changes in the solubility of fat components throughout this procedure. Dry fractionation, solvent fractionation, and molecular distillation are processes that can be used in fat synthesis based on differences in molecular mass, melting temperature, volatility, and intermolecular interactions among triacylglycerol. Short, medium, and long-chain triacylglycerol fractions of high purity can also be obtained by supercritical carbon dioxide extraction. As a more neutral method, dry fractionation without solvents is preferred. During the process, the target crystallisation temperature and cooling rates are monitored. Temperature fluctuations and a wide range of melting and solidification temperatures allow for the extraction of fractions with varying compositions and qualities (Malkowskaet al. 2021). In the food processing sector, the obtained fractions are employed for a particular purpose. Solid fractions are mostly utilised in the preparation of cakes and pastes as additions. In tropical regions, they are also used as hardening agents in the manufacturing of ghee and recombinant butter. Butter spreadability is improved by liquid fractions. Aromatic chemicals, pigments, cholesterol, and vitamin A are abundant in liquid fractions, which are used as functional food additives (nutraceuticals) in the baking, confectionery, chocolate, and dairy sectors. Chocolate fat blooms are prevented by high-melting milk fat fractions. Ice cream and processed cheese texture are improved by using low-melting milk fat fractions (Malkowskaet al. 2021) to adjust the profile. The physical form of the product can be changed to lower cholesterol from milk fat. Adulterants such as vegetable oil (palm oil, groundnut oil) and animal body fat (goat body fat, sheep body fat) is also detected using the fractionation technique.

Solvent fractionation provides advantages over dry fractionation in that, in addition to lowering the viscosity of the liquid, it facilitates heat transfer, accelerates nucleation and growth, and has very low levels of entrained oil. Sheep body fat and palm olein, both of which are less expensive, are suspected of being utilised as adulterants in milk fat (Gandhi,2014).Different studies have been conducted using fractionation to detect adulteration have been discussed in this paper.

**Studies on the fractionation of ghee**

To fractionate the ghee, the crystallization method was used. Ghee was heated to 60°C to eliminate the crystal memory. After that, it was gradually cooled to 30°C in an incubator for 12 hours to crystallise. Decantation was used to separate the liquid from the crystals after centrifugation at 2000 rpm for 10 minutes in a temperature-controlled centrifuge held at 30°C. The solid fraction obtained (S30) was considered a high melting fraction at 30°C. The liquid fraction obtained at 30°C was then incubated at 20°C for another 12 hours. The crystals that were formed were separated. The solid proportion at 20°C was referred to as the medium melting fraction (S20), meanwhile the portion that remained liquid at 20°C was referred to as the low melting fraction (L20) (Kankare 1974).

Thirty grams of melted ghee (pure and samples containing a mixture of adulterants at (0 percent, 10%, 20%, 30%) were placed in a 100 mL graduated glass tube and equilibrated at 65°C for 5 minutes in a temperature-controlled oven, and 60 mL of acetone was added to the tube to give a ghee-to-acetone ratio of 1:2 (w/v). It was maintained at 50 °C for 5 minutes after completely mixing to equilibrate. Following that, fractionation was done in a chilled water bath at two temperatures: (15°C for 15 minutes, followed by 4°C for 2 hours) To remove the entrapped acetone, the solid fractions were held in a temperature-controlled oven at 110 °C for about 1 hour, while the liquid fractions were first subjected to rotating vacuum evaporation at about 40 °C, followed by heating to 110 °C for about 1 hour. Finally, all of the melted fractions were nitrogen flushed to

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ensure complete solvent evaporation (Gandhi et al. 2014).

Ghee was made from triple fractionated buffalo milk (BMG) was melted at 65°C for 15 minutes to eliminate crystals. Separation of milk fat fractions was performed in a shaking incubator with an agitation speed of 80 rpm and a cooling rate of 5 °C/min, with fractionation temperature only changing for unique milk fat fractions. To stabilize fat crystals, the crystallization temperature was kept at 25 °C for the first 20 hours. Centrifugation was used to separate the resultant fractions, which were termed SBMG 25 (solid fraction) and LBMG 25 (liquid fraction). The second fractionation phase proceded with fraction LBMG 25, and the rest of the method was repeated, with crystallization at 20°C. The fat fractions obtained in this phase were SBMG 20 (solid fraction) and LBMG 20 (liquid fraction). The fraction LBMG 20 was used in the third fractionation using the same method as before, but with a crystallization temperature of 15°C. The fat fractions recovered in this fractionation step were SBMG 15 (solid fraction) and LBMG 15 (liquid fraction). The fractions LBMG25, LBMG20, and LBMG15 were adopted for the study (Mudgilet al. 2020).

Fractionation butter oil was heated for 10 minutes at 70 °C then gradually cooled to 35°C in a circulating water bath. Centrifugation at 5000 rpm for 5 minutes separated the solid fraction (S35) from the liquid fraction (L35) at 25 and 15°C, providing four fractions: the solid fraction (S35, S25, and S15) and the liquid fraction (L15) (Abbas et al. 2020).

Ghee was completely melted in the incubator at a temperature of 60°C. The process was carried at 30°C for 72 hours, 25°C for 72 hours, and 20°C for 72 hours. Milk fat was fractionated in the incubator. At each temperature (30°C, 25°C, and 20°C), solid fractions were separated from liquid fractions using filter paper (Whatman) (Malkowska et al. 2021).

**Detection of adulteration using solvent fractionation with Butyro-Refractometer (B.R.) reading**

The solvent fractionation process is used to produce various fractions enriched in animal body fats or vegetable oils, which are then used to estimate butyro-refractometer (B.R.) readings. The solid fraction issupplemented with animal body fats, while the liquid portion is supplemented with vegetable oils. Pure cow ghee has a B.R. reading ranging from 41.57 whereas pure buffalo ghee has a B.R. reading ranging from 40.72 palm olein and sheep body fat added individually cannot be detected at even 15% levels in pooled cow and buffalo ghee samples. Only at 9+21 (30%), the percent level was a blend of palm olein and sheep body fat detectable. After fractionation, however, only 3+7(10) and 6+14 (20%) were detectable (Gandhi et al.2017).

**Detection of adulteration using solvent fractionation with Reichert-Meissl (RM) value**

For pure cow ghee, the RM value ranged from 28.60 to 30.36, with an average value of 29.50, while that for pure buffalo ghee ranged from 31.46 to 34.98, with an average value of 33.30. When palm olein and sheep body fat was added separately, they could only be detected at 15 percent levels in combined cow and buffalo ghee samples. A mixture of palm oil and sheep body fat was detected at levels of 6+14 (20) and 9+21 (30). Fractionation, however, allowed even lower levels of 3+7 (10%) to be detected that could not be detected before. (Gandhi et al. 2014).

**Detection of adulteration using solvent fractionation with Iodine value**

As for pure cow ghee, the iodine value ranged from 35.53 to 41.24 with an average of 38.69, whereas that for pure buffalo ghee was 30.14 to 36.48 with an average of 34.10 Based on the iodine values, palm olein and sheep body fat added individually could not be detected at any level in pooled samples of ghee. A mixture of palm olein and sheep body fat was detected only at 9+21(30) percent. A lower level of 6+14(20) percent was also detectable after fractionation (Gandhi et al. 2015).

**Detection of adulteration using solvent fractionation technique coupled with Apparent solidification time (AST)**

When palm olein and sheep body fat were added separately and in combination with ghee, the apparent solidification time (AST) test (min: sec) was utilized to detect adulteration. The palm olein/sheep body fat mixture was detected at a 9+21 percent level. With the use of fractionation, the lower level of 6+14 percent was also found to be detected. Thus, when combined with AST, the solvent fractionation technique increases the sensitivity of the AST Test and could be used as a helpful measure for detecting adulteration of palm olein and sheep body fat in cow and buffalo ghee. (Gandhi et al.2018).

**Detection of adulteration using Solvent fractionation technique coupled with Complete Liquefaction Time(CLT)**

The complete liquification time (CLT) test is performed when solid fat melts completely at a

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specified temperature within a set period of time. Added singly or combined with ghee, it has been used to detect adulteration of groundnut oil and goat body fat. It was found that when adulterants are added separately, they are detectable at higher levels (15%, w/w, groundnut oil in cow ghee and 10%, w/w, goat fat in buffalo ghee), while fractionation reduces the detection limit to the lowest level (10%, w/w) used in this study (Upadhyay et al. 2017).

Pure cow ghee had a CLT value (min: sec) ranging from 2:13 to 2:53, with an average of 2:32, whereas pure buffalo ghee had a CLT value (min: sec) ranging from 2:22 to 3:08, with an average of 2:43. The palm olein and sheep body fat mixture could be detected at the levels of 6+14 (20) percent and 9+21 (30) percent, but not at the level of 3+7 (10) percent. A fractionation result showed that even a lower level of 3+7 (10) percent was noticeable (Kamal et al. 2018).

## II. CONCLUSION

Fractionation could be used as a tool to detect adulterants (vegetable oil and animal body fat) in ghee. It helps in decreasing the detection limit and increased the sensitivity of iodine value, Butyro-Refractometer (B.R.) and Reichert Meissl (R.M.) value, Apparent solidification time (AST),Complete Liquefaction Time (CLT).

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