Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2024; 13(5): 240-246
Received: 02-07-2024
Accepted: 03-08-2024

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Comparative assessment of ghee residue from two different sources: An exploratory study towards utilization of ghee byproducts in alignment with zero-waste principles

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DOI: https://doi.org/10.22271/phyto.2024.v13.i5c.15078

Abstract
The present study focuses on a comparative assessment of ghee residue (GR) sourced from two types of milk—hybrid cow (HGR) and desi cow (DGR). Ghee residue being a potential source of valuable nutrients is often overlooked despite of numerous established research evidences. This byproduct produced during the ghee manufacturing process is underutilized and discarded as waste. This research evaluates the physicochemical properties of HGR and DGR to understand their potential for various applications. The values for moisture content, ash content, protein content, fat content, Reichert Meissl, Polenske, and total phospholipid content were more prominent in the case of hybrid cow ghee residue as compared to desi cow ghee residue. The results revealed notable differences between HGR and DGR, particularly in moisture and fat content. HGR exhibited a higher moisture content (12.30%) compared to DGR (8.23%), making it more susceptible to microbial contamination. However, the fat content in HGR (35.94%) was higher than in DGR (32.43%). Both residues displayed comparable protein content (around 18%) and phospholipid profiles. Sensory attributes such as color and texture also differed, with HGR showing a caramel cream color and a caky texture, while DGR was darker and more flow. Thin layer chromatography of the isolated phospholipids from both sources was done to analyze the number of phospholipid components present. The findings highlight the need for standardizing ghee residue processing methods to maximize its use, thereby reducing waste and environmental impact while creating new economic opportunities complying with the zero-waste principles.

Keywords: By-product, ghee residue, sensory attributes, phospholipid content, thin layer chromatography

Introduction
Waste management at the industrial scale is always a cumbersome and challenging task leading to extra burden on the industry which ultimately causes increased production cost. With the principles of 4Rs in waste management i.e. Reduce, Reuse, Recover and Recycle along with an approach of Zero Waste; this extra burden can be minimized dramatically. One of such approach is exploring possibilities of utilizing waste for some other purpose considering it as a 'byproduct' instead of 'waste'. Byproducts are described as the additional products produced or the material remained during the manufacturing of a significant product and the industries have traditionally emphasizing on economic utilization of these by products. The use of by-products benefits the industry's economics by boosting production capacity and product diversification while also reducing the strain on effluent treatment plants (ETPs) to reduce the organic load in effluents before disposal. This is in line with the European Union's (EU) action plan for 'circular economy' to minimize food waste and encompasses a comprehensive approach to reduce, reuse, recover, and recycle nutrients and energy [1].
This manuscript is based on the byproduct generated during ghee production in India. Ghee ranks second after milk in most widely consumed dairy foods, in India. *Ghee* (clarified butter fat) is a fat-rich dairy product having widespread popularity and applications in manufacturing various products, frying and even for direct consumption [2, 3]. Ghee preparation invariably results in the yield of ghee residue (GR) as a byproduct. The GR is scientifically referred to as Clarified Butter Sediment Waste (CBSW) since it is produced as sediment during the butter fat clarification process. The GR is a dark-brown byproduct that consists mostly of the solid not fat (SNF) and the entrapped fat. Although the amount of GR gained during the process varies depending on the raw material used for ghee preparation, it looks to be approximately 10% of the ghee produced [4].

According to Nationmater.com (2021), India and the United States are the leading ghee producers, with five-year compound annual growth rates (CAGRs) of +3.60% and +3.50%, respectively. Given the amount of ghee generated in 2020 (1,70,000 metric tons), India produced around 17,000 metric tons of GR, which is a significant amount. The GR is regarded as an excellent vendor of critical nutrients. It is mostly composed of residual fat (what remains after fat extraction), milk proteins, lactose, and minerals.

Despite limited information on the efficient utilization of ghee residue, much of the industrially produced ghee residue is often discarded into drainage systems. Although the use of ghee residue presents various challenges, it also offers significant opportunities for effective utilization. To realize and assess the full potential of GR, it is critically essential to address nutritional and safety concerns, standardize processing methods, enhance economic feasibility, and advance technological capabilities. The coordinated efforts in research, regulation, and market development are required for yielding benefits for both the economy and the environment by which the value of GR can be maximized. Though currently it is underutilized, recent studies and industry practices in India underscore innovative applications and an increasing recognition of its value as evident in the literature [5].

Utilizing ghee residue enhances economic value, environmental sustainability, and industrial innovation. The dairy industry can transform this byproduct into profitable products due to GR's rich nutritional profile, which includes fats, proteins, and antioxidants, making it valuable for food items like bakery goods and dairy products. This not only adds value but also meets the demand for nutrient-dense foods. Proper utilization of GR reduces waste and environmental pollution, significantly lowering the industry's environmental footprint and promoting a circular economy. Beyond food, GR is used in producing industrial enzymes like lipases and as a feedstock for biodiesel, offering an eco-friendly fuel alternative. Additionally, GR can be repurposed as animal feed, organic fertilizer, and in cosmetic and personal care products. It also holds potential in pharmaceuticals for nutraceuticals and dietary supplements. Research and innovation are crucial for expanding GR's utility, with processing advances mitigating limitations and driving new products and processes that maximize its benefits. In short, ghee residue utilization is essential for achieving a sustainable and innovative future in the dairy industry [6].

Ghee residue is mainly generated during ghee manufacturing, a step-by-step process that varies depending on the method of manufacture employed. The traditional method involves five stages, known as *Samskara*, which include procurement and boiling, curdling, churning, separating butter from buttermilk, and final processing. This method is considered as the scientific process for making high-quality ghee in small quantities according to Ayurveda. There are few more ghee manufacturing processes mostly used by the home makers and commercial manufacturers that include direct cream method, creamery butter method, pre-stratification method, and continuous method. Each method has its own specific steps and requirements; however, the most commonly used methods are direct cream method and creamery butter method, amongst which creamery butter method is preferred for commercial production due to its higher yield while ghee residue content is higher in the direct cream method [7]. The composition and quality attributes of ghee residue may vary depending on the source and manufacturing process. Analyzing the physicochemical differences in ghee residue from various sources can provide insights into the manufacturing process and help to optimize the production of high-quality ghee and its byproducts. Concisely, the comparative evaluation of the physicochemical properties of ghee residue from different sources was imperative in understanding composition and quality differences, optimizing utilization and applications, improving manufacturing processes, and ensuring regulatory compliance. In the present project, comparative analysis of GR obtained from two different commercial producers of ghee out of which one produces ghee from the milk obtained from hybrid cows while other from the milk of desi cows. This study would help us to minimize the challenges and provide a comprehensive understanding of the current state and potential solutions for the effective use of ghee residue for minimizing waste and to protect environment.

Materials and Methods

- **Materials:** The samples of ghee residue were collected from two different sources commercially well established and known for their ghee production. Hybrid ghee residue (HGR) was collected from, Jai Bhole Dairy, Nagpur, while desi ghee residue (DGR) from, GoVigyan-Anusandhan Kendra, Deolapar, Nagpur. The first one produces ghee mainly for the direct consumption purposes whereas, second one for direct consumption and the medicinal preparations of Ayurvedic origin. Creamery butter method of ghee production was employed at both the sources of ghee residue.
- **Methods:** The residue was collected immediately after ghee production and passed through sieve no. 40. For further studies ghee residue was refrigerated in air-tight containers.

Physicochemical Parameters

The preserved ghee residue was then subjected to complete analysis i.e. Moisture, ash, and fat content as per AOAC (2003) [8]. The protein estimation was done by Lowry method. The sensory attributes of the ghee residue were evaluated based on color, odor, and texture.

Moisture Content: Approximately 5 g of each HGR and DGR was weighed accurately, the initial weight was recorded and transferred to properly dried petri plates. The samples were then placed in a drying oven, and the temperature was set to 105 °C. The samples in the petri plate were allowed to dry for a specified period of 24 h and allowed to cool in a desiccator to avoid moisture absorption. Once cooled, the samples were reweighed, and the final weights were recorded. The moisture content was calculated using the formula:

$$\text{Moisture Content (\%)} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \text{ X } 100$$

Ash Content

About 5 g each of HGR and DGR was weighed accurately and finely grounded in order to get a uniform size. The crucibles were cleaned, dried, weighed, and the weights were recorded. The ground samples were placed into the crucibles. The crucibles containing the samples were placed in a muffle furnace set to a temperature of around 550°C and kept for 4-6 h until turned into a consistent grey or white ash. The crucibles were removed from the furnace and had been

allowed to cool in a desiccator to prevent moisture absorption. After cooling, the crucibles with the ash residue were weighed, and the ash content was calculated using the formula:

$$\text{Ash Content (\%)} = \frac{\text{Weight of Ash}}{\text{Initial Weight of Sample}} \times 100$$

Crude Fat

About 50 g of HGR was weighed accurately and transferred to a thimble in Soxhlet extractor. The Soxhlet extraction apparatus was assembled, ensuring all components, such as the condenser, extractor, and flask, were properly connected. Petroleum ether was poured into the Soxhlet apparatus until the desired level was reached and the extraction process was initiated by heating the solvent to reflux. The solvent was repeatedly siphoned over the sample, dissolving the fats, and returning to the boiling flask. This extraction process was continued for a specified period, usually 4-6 h, ensuring complete fat extraction from the sample. After the extraction, the solvent containing the dissolved fat was evaporated by heating the flask on a rotary evaporator or under reduced pressure until only the crude fat remained. The crude fat residue was further dried in an oven at a specified temperature of around 100°C, to remove any remaining solvent. The dried fat residue was cooled in a desiccator to room temperature and then weighed accurately using a balance. The crude fat content was calculated by dividing the weight of the dried fat residue by the initial weight of the sample and multiplying it by 100 to obtain the crude fat percentage. The procedure was simultaneously performed for DGR to determine the fat content.

Crude Protein

Lowry method: Different dilutions of Bovine Serum Albumin (BSA) were prepared by mixing 1 mg/ml with distilled water. Accurately measured (pipetted) 0.2 ml of each of the above dilutions 2 ml of alkaline copper sulphate reagent was added and mixed thoroughly followed by incubation at room temperature for 10 min. To each of these dilutions, 0.2 ml of Folin Ciocalteau reagent was added and further incubated for 30 min. Absorbance for all the dilutions was recorded at 660 nm and the standard curve was plotted. The unknown sample of the HGR and DGR were prepared by diluting 1 mg in 10 ml of distilled water. The solution was sonicated for 2 min, filtered for further dilution and 0.2 ml of this solution was processed in the similar manner as that for BSA solution. The absorbance of the unknown sample was recorded and the concentration was determined using the plotted standard curve.

Reichert Meissel Value and Polenske Value

The Reichert-Meissl (RM) and Polenske values are important parameters used to analyze and characterize fats and oils, especially in dairy products. The RM value indicates the amount of volatile, water-soluble fatty acids (mainly butyric acid) that can be extracted from fat through saponification. In contrast, the Polenske value measures the amount of volatile, water-insoluble fatty acids (mainly caproic, caprylic, and capric acids) that can be extracted from fat through saponification (method of detection of foreign fats in the dairy product). The RM value and Polenske value are important analytical tools used in the dairy industry to characterize fats, detect adulteration, and ensure the quality and authenticity of dairy products like butter and ghee. A sample of 5 g of ghee residue was weighed accurately and saponified by heating it with 20 ml of 0.5 N alcoholic potassium hydroxide solution for 30 min. The saponified mixture was further acidified with 40 ml of 0.5 N sulfuric acid to release the fatty acids. The volatile fatty acids were distilled off by adding 110 ml of distilled water and using steam distillation for 60 min. The distillate containing the volatile fatty acids was collected in a 100 ml volumetric flask and titrated with 0.1 N sodium hydroxide solution using phenolphthalein as an indicator. The RM value was calculated based on the volume of sodium hydroxide used for titration, representing the amount of volatile fatty acids in the ghee residue.

Around 5 g of ghee residue was accurately weighed and saponified by heating it with 25 ml of 0.5 N alcoholic potassium hydroxide solution for 30 min. The saponified mixture had been acidified with 40 ml of 0.5 N sulfuric acid to release the fatty acids. The volatile fatty acids were distilled off by adding 100 ml of distilled water and using steam distillation for 90 min, leaving behind the non-volatile fatty acids. The remaining mixture was cooled and the non-volatile fatty acids were extracted with 50 ml of petroleum ether. The petroleum ether layer containing the non-volatile fatty acids were evaporated to dryness. The residue was dissolved in 50 ml of 95% ethanol and titrated with 0.1 N sodium hydroxide solution using phenolphthalein as an indicator. The Polenske value was calculated based on the volume of sodium hydroxide used for titration, representing the amount of non-volatile fatty acids in the ghee residue.

Total Phospholipid Content

The Folch method was employed for lipid extraction (9). This process involved the usage of a chloroform-methanol mixture (1:1 v/v) to extract lipids from the ghee residue. The extracted lipids were subjected for determining phospholipid content employing UV spectrophotometric methods using ammonium molybdate with Potassium dihydrogen phosphate as standard. The composition of phospholipids was analyzed using thin-layer chromatography (TLC).

Briefly, about 1 g of ghee residue was weighed and transferred to 50 ml centrifuge tube followed by shaking with a mixture of 10 ml each of chloroform and methanol for extracting phospholipids. Further, the mixture was centrifuged at 2000 rpm for 10 min to separate the phases. The lower chloroform phase was collected, dried and preserved for studies. The dried extract was added with 10 ml each of H_2SO_4 and HNO_3 and digested until a pale shade of yellow was developed. In an aliquot portion (5 ml) from the digested sample, 0.44% ammonium molybdate and 0.4 ml of reducing agent (Equal amount of 0.2% w/v of 1-amino-2-naphthol-4 sulphonic acid, Sodium sulphite, Sodium bisulphite) were added and the flask was immersed in boiling water bath for 7 min. The optical density of the sample was read at 720 nm using a UV spectrophotometer. Potassium dihydrogen phosphate (1 µg/ml) was used as standard, whereas 0.5% H_2SO_4 was used as blank for this analysis (10, 11).

Thin Layer Chromatography (TLC)

The chloroform extract was diluted and sample was prepared for single spot application on a TLC plate of size 10*2 cm. The mobile phase comprising solvent system of chloroform: methanol: ammonia solution (28% v/v) (65:25:4) was kept in a TLC chamber allowing the chamber to saturate for 15 min. The TLC plate was developed and the phospholipid bands were visualized using an iodine vapor. The phospholipid

components were confirmed by comparing their R_F values with known standards [12, 13].

Results and Discussion

The sensory attributes of the HGR & DGR based on color and texture were different whereas the odor was same. The color of HGR was caramel cream and the texture was caky while the color of DGR was dark brown and the texture was flowy. This was observed due to varied heating and pressing time during and after the ghee manufacturing. Sensory evaluation is essential for understanding how consumers perceive ghee residue products as it helps in refining product formulations to enhance desirable attributes while minimizing any off flavors or undesirable textures. This process is critical for developing marketable products that utilize ghee residue effectively, turning what is often seen as wasted to high value food items. In summary the sensory attributes of ghee residue and its derivatives significantly influence consumer preferences and acceptance making them a vital consideration in product development and optimization.

All analytical experiments were conducted five times (n = 5), and results are represented as mean values with standard deviation. The significance of all factors was evaluated at a p-value threshold of 0.05. Proximate analysis is a common method used to determine the nutritional composition of food and other organic materials. It involves breaking down a substance into its basic components to analyze its content. However, ghee residue may not have uniform results and it completely relies on the source of ghee residue and method of ghee manufacturing.

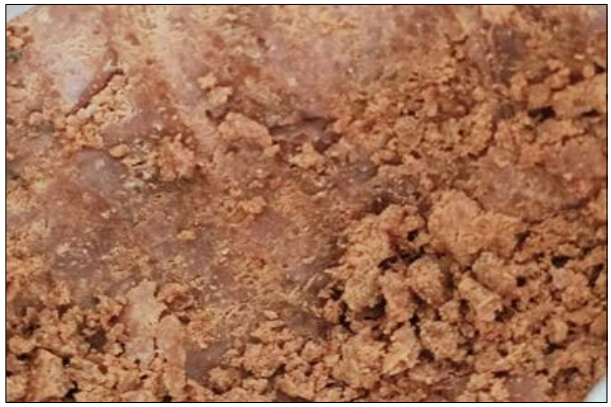


Fig 1: Hybrid ghee residue



Fig 2: Desi ghee residue

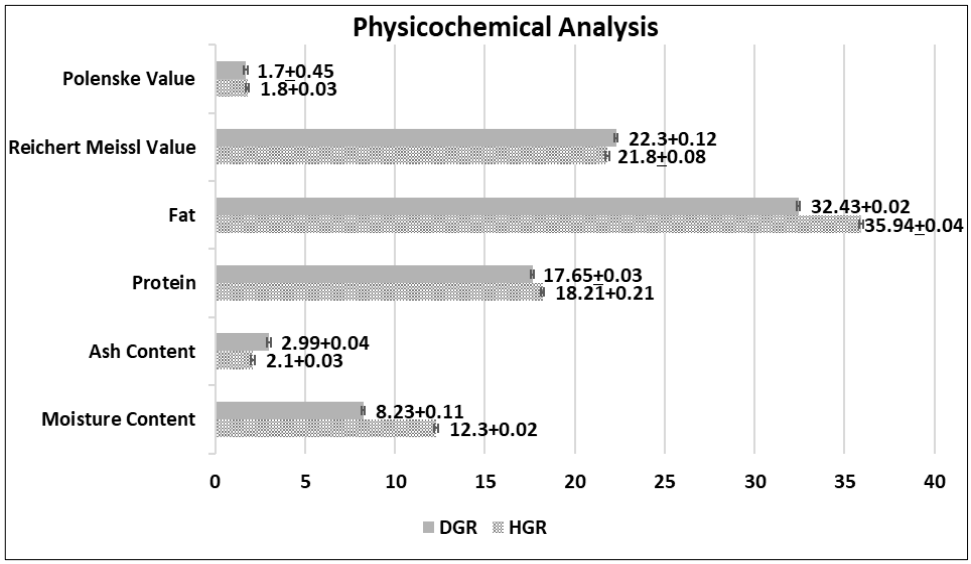


Fig 3: Graphical representation of physicochemical analysis of ghee residue

Moisture content in the HGR was found to be 12.30 %, which is high compared to DGR with 8.23%. This variation has revealed the nonuniformity of the filtration time after the ghee manufacturing process. In addition, the heat treatment employed for the serum solids for ghee production plays a crucial role in the moisture content of ghee residue. As in the process of ghee production, the butter is heat treated to 110-120 ˚C for 10-20 min for complete moisture removal thus resulting in 0.10-0.20 % moisture content in the produced ghee. But on either side, the moisture content in ghee residue has been reported to be in the range of 12.10 to 26.64% [14]. This suggests that some of the moisture is not completely removed from the contents during the boiling stage of the ghee and that GR functions as a water-binding agent to extract any remaining moisture from the heat-treated fat phase (ghee). Protein and other water-soluble milk solids may be the cause of the water-binding characteristic.

Moisture content in ghee residue makes it vulnerable to microbial infection. Strict cleanliness and preservation procedures are needed to guarantee its safety for ingestion or use in animal feed.

Moisture content and ash content are both important parameters that can provide insights into the properties and quality of a material. Moisture content refers to the amount of water or other volatile substances present in a sample, while

ash content represents the inorganic residue that remains after the complete combustion of the organic matter.

Generally, there is an inverse relationship between moisture and ash content. As the moisture content of a sample increases, the ash content tends to decrease. This is because the presence of water in the sample dilutes the concentration of the inorganic components, resulting in a lower ash content.

The ash content for HGR and DGR were found to be 2.10 % w/w and 2.99% w/w, much lower than the ash content range of 4.83% w/w to 5.2% w/w observed by Loganathan (2015) [15]. This proves how the source of GR affects the physicochemical parameters.

The protein content in HGR and DGR was observed to be 18.21% w/w and 17.65% w/w which doesn't show any significant variation. Depending on the source material and procedures used to make the ghee, the protein concentration in the GR might range from 18.60 % w/w to 33.50% w/w. GR made from raw materials with lower SNF content, such as creamery white butter, has a lower protein level than SNF, such as cream. Furthermore, the heat treatment that occurs during the boiling of ghee frequently results in modifications to the quality of the protein. The soluble nitrogen content was found to decrease from 0.89 to 0.38 % w/w when the heat treatment was increased from 110 to 120 °C. This was mainly due to the heat-induced denaturation of milk proteins [16].

The fat content of HGR and DGR was found to be 35.94% w/w and 32.43% w/w. The fat content of the raw material did not seem to be directly correlated with the fat content of the GR, in contrast to yield. This is partially consistent with the findings of Pal *et al*. (1975) [17], who reported that a decrease in fat losses in the GR is proportionate to a rise in the fat content of the raw material. It was found that when raw material fat content rises, there is a corresponding decrease in GR and, consequently, in the amount of materials that may trap and retain GR fat. However, the amount of pressure applied to remove the fat from the GR (when squeezed in the fine cloth) may also have contributed to the increased fat content in the GR samples; that is, more pressure will extract more fat from the GR and leave less fat in the final GR.

The RM value of HGR and DGR was found to be 21.8 and 22.3, indicating the amount of volatile fatty acids, primarily butyric acid, in ghee residue samples. RM value measures the degree of hydrolytic rancidity, which is the breakdown of the fats due to water exposure and enzymes. Higher RM values in DGR indicate higher levels of volatile fatty acids and thus greater susceptibility to rancidity as compared to HGR, however, the RM values for both the samples were found to be in the acceptable range i.e. 17-30. These values play a crucial role in assessing the stability and shelf-life of ghee residue. The degree of refinement or purification of ghee residue was assessed by the Polenske value which is another measurement of the amount of volatile fatty acids, mainly caproic and caprylic acids, produced by acid hydrolysis of the fat. The PV value of HGR and DGR was found to be 1.8% and 1.7%, relating to the quality of fats, particularly those used in food. Lower PV values suggest better quality and purification of the fat present in the sample. Assessment of PV determines the purity and appropriateness of fats for various uses, including food processing, when frequently used in conjunction with other tests [18].

The above-discussed physicochemical properties reveal that the method used to prepare ghee and the milk's source can have a significant impact on the quality of the residue. Its large-scale use and standardization are hampered by this inconsistency. Standardized procedures for the management, preparation, and application of ghee residue are lacking. This makes it more difficult to develop reliable procedures and products out of the ghee residue.

Calculating the total phospholipid content from phosphorus analysis involves converting the measured phosphorus concentration to phospholipid concentration using a conversion factor. This conversion factor depends on the assumption that phospholipids contain a known amount of phosphorus within their molecular structure. The total phospholipid content in both the ghee residues has not shown any significant variation as the ghee manufacturing process of both sources was same.

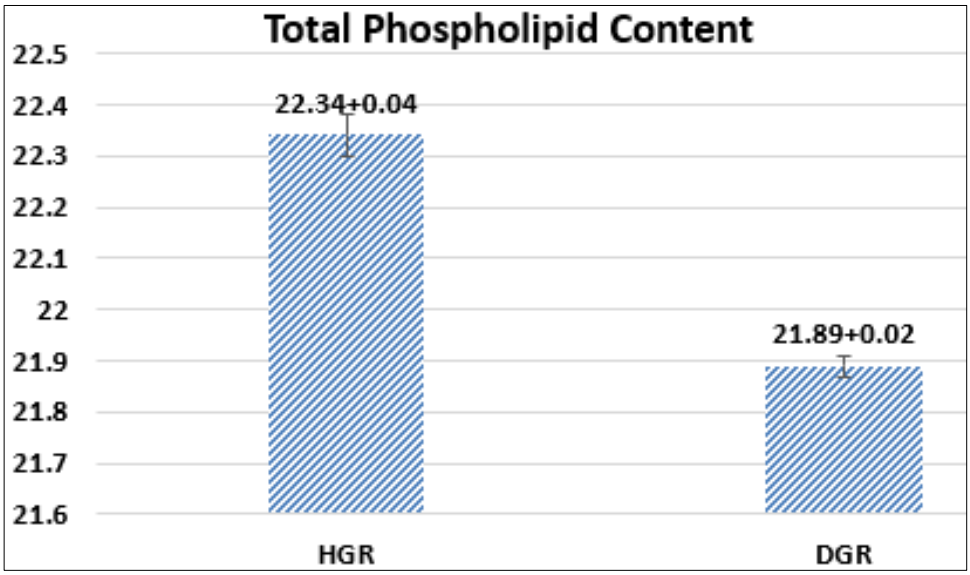


Fig 4: Graphical representation of total phospholipid content

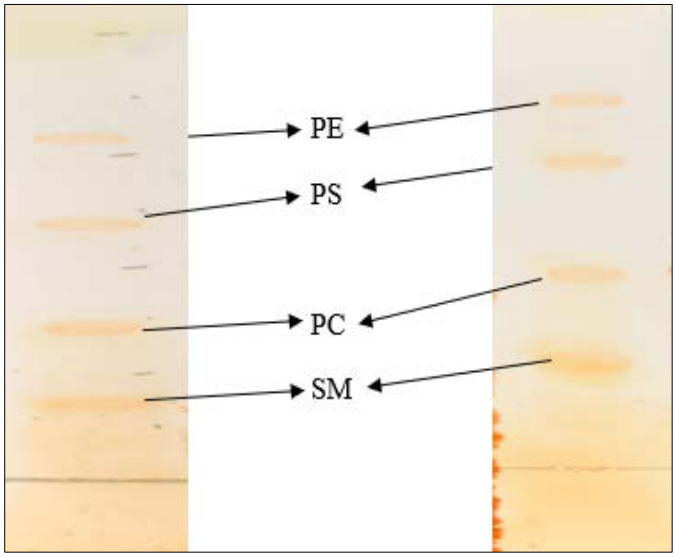


Fig 5: TLC of HGR & DGR

Table 1: Comparative R_F values of various classes of phospholipids

Phospholipid class	Standard	HGR	DGR
Phosphatidylcholine (PC)	0.41	0.38	0.42
Phosphatidylethanolamine (PE)	0.91	0.88	0.90
Phosphatidylserine (PS)	0.78	0.69	0.74
Sphingomyelin (SM)	0.15	0.18	0.21

Figures 5 shows the one-dimensional thin-layer chromatography of HGR and DGR with individual single spots of various components with varying R_F values as mentioned in Table 1. As the study deals with the whole phospholipid content from the ghee residue, various classes of phospholipids were depicted through different bands on the TLC plate. These various classes of phospholipids include Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylserine, and Sphingomyelin.

The R_F values for the standards of various classes of phospholipids were referred from the data reported by Pragasam *et al.* and Seunghyun *et al.* [19, 20]. The ratio of the distance travelled by the solvent (front) to the distance travelled by the spots was used to calculate the R_F values for each spot, and these values were then compared with the standards.

The average R_F values of components of HGR and DGR are close to the standard R_F values of of PC 0.41, PE 0.91, PS 0.78, and SM 0.15. The sample's R_F values have an exact correlation with the standard and possess a minimal deviation and acceptable precision.

Conclusion

The comparative assessment of ghee residue from desi and hybrid cow ghee revealed significant differences in their physicochemical properties demonstrating that hybrid cow ghee residue (HGR) exhibited higher moisture, protein, and fat content compared to desi cow ghee residue (DGR), making it a more nutrient-rich byproduct. However, the higher moisture content in HGR makes it more prone to microbial contamination, suggesting the need for proper handling and preservation methods. Both residues showed similar phospholipid profiles, indicating comparable functionality in terms of lipid content. The sensory attributes differed in color and texture, which may influence consumer acceptance of food-related applications.

Despite challenges like inconsistency in physicochemical properties based on the source and production methods, the findings highlight the need for standardizing ghee residue processing methods to maximize its use, thereby reducing waste and environmental impact while creating new economic opportunities aligning with zero-waste principles by reducing industrial waste and promoting sustainable practices. Future work should focus on developing optimized processes for large-scale utilization and exploring the residue's role in promoting environmental sustainability and economic viability.

Author Contributions

AD: Methodology, Investigation, Validation, Writing – original draft. HM, and Formal analysis, Resources, Validation, Drafting of MS, Writing – review & editing. SM: Data curation, Writing – review & editing. NR & PK: Conceptualization, Methodology, Validation, Investigation and finalizing manuscript.

Conflict of Interest Statement

Authors declare no Conflict of Interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Acknowledgement

First author gratefully acknowledges the financial support from Dr. Babasaheb Ambedkar Research and Training Institute (BARTI) for providing financial support for the research and publication of this article in the form of BANRF Fellowship for the Ph. D. research work, which helped us complete this work successfully. (Fellowship Award Ref No. BARTI/Fellowship/BANRF-2018/19-20/3036 Dated 30-06-2020).

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