

Recovering Phospholipids from Ghee Residue (a dairy industry by-product) Using Emerging Technologies

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Introduction

Some of the by-products of the dairy industry include ghee residue, cheese whey and paneer whey. Among these, ghee residue is a nutrient-rich, brownish solid mass obtained as a by-product during the preparation of ghee in the dairy industry. It contains 4–13% moisture, 33–65% fat, 18–42% protein, 0.5–15% lactose and 2–5 % minerals (Singh, 2014). Phospholipids (3.6 to 17.39 g/100 g), vitamin A and E, amino acids, proteins with free sulphhydryl groups, and protein-carbohydrate complexes exhibiting antioxidant characteristics (Santha and Narayanan 1978; Singh *et al.*, 1979; Lal *et al.*, 1984) are also present in it. Among these, this article focuses on phospholipids, which possess antioxidant, antimicrobial, and antiviral properties. Studies have also established that they contribute to reducing the risk of Alzheimer's, stress, and memory decline, or implication in other mental disorders such as depression. Most of these health benefits are attributed to the presence of polyunsaturated fatty acids in their sn-2 position (Pimentel *et al.*, 2016).

Apart from the biological functions, phospholipids also have techno-functional advantages owing to their synergistic behaviour with the reducing compounds present in ghee residue, thereby having the potential of preventing oxidative defects and enhancing the shelf life of food products. The bipolar nature of phospholipids makes them very good natural emulsifiers. Thus, ghee residue extract rich in phospholipids could be potentially beneficial in developing food products where emulsification of fat in aqueous phase is a desirable characteristic, such as dairy spreads and ice cream mixes.

Extraction of phospholipids from dairy sources

Before the realisation of the high value of phospholipids for isolation, efforts were focused on enriching ghee with phospholipids by using the solvent extraction method, resulting in their transfer into ghee from the ghee residue. Pruthi *et al.* (1973) proposed solvent extraction methods and a heat processing method to recover phospholipids from ghee residue. Various organic solvents were used in the solvent extraction method to drive phospholipids from ghee residue. By using a controlled heating technique, phospholipids are directly extracted from

ghee residue to ghee. Later, Lal *et al.* (1984) standardised the method of transferring phospholipids from ghee residue to ghee by two kinds of solvent extraction. In the case of the solvent extraction method, chilled acetone (4–5°C) was used to wash ghee residue thrice, and washings were discarded. With an equal quantity of ethyl alcohol, the residue was added, followed by warming to 50–60°C followed by cooling to room temperature. Lipids in the residue were extracted using ethyl ether and petroleum ether thrice, and extracts were dried in vacuum. In this method, ghee residue was washed with acetone followed by extraction with warm alcohol. Similar to the first case, the alcohol extraction was carried out three times, followed by drying in vacuum.

After obtaining crude phospholipids in both cases, they were subjected to purification as follows: the dried lipids were dissolved in ethyl ether (minimal quantity), and phospholipids were precipitated with acetone at 5°C. The precipitation of phospholipids with acetone was repeated for 6 times. Phosphorus content was estimated to assess the purity of phospholipids. Further, to ascertain different fractions of phospholipids present, they were subjected to fractionation on silica gel thin-layer liquid chromatography. Results in the above method of extraction revealed that the purity of phospholipids obtained after two methods of extraction varied widely. Average % purity in the first method reported 57.6% whereas the second method yielded 48.0% purity. Overall, the researcher concluded that solvent extraction methods are tedious and pose difficulties in removing the solvent.

Advances in extraction of phospholipids

Now that there is an understanding of the beneficial role of phospholipids in terms of their health benefits and their functionality as an emulsifier, there is an interest in extraction, quantification and purification of phospholipids sourced from ghee residue.

Microfiltration of buttermilk has been identified as an effective method for concentrating the milk fat globule membrane (MFGM). The concentration of phospholipids can be enhanced using buttermilk derived from washed cream (Morin *et al.*, 2007). Adding sodium citrate to disrupt micellar caseins reduces casein contamination from 30% to 60% of the

total protein, thereby increasing the MFGM proportion in the isolates (Corredig et al., 2003). Additionally, combining ultrafiltration with hydrolysis is believed to further boost the phospholipid content in the extracts (Konrad et al., 2013).

Microwave-assisted extraction (MAE) causes the disorder or modification of cell structures through application of non-ionizing electromagnetic waves (Leonelli et al., 2012). Energy transfer in MAE occurs through two main mechanisms: ionic conduction and dipole rotation, which often occur simultaneously in most applications. Ionic conduction involves the movement of ions through a solution, generating uniform heat in the medium due to the solvent's resistance to ionic migration when electromagnetic waves are applied (Veggi et al., 2013). Dipole rotation refers to the reorientation of molecules in the direction of the electric field, causing thermal agitation when the electromagnetic waves are removed as the molecules return to a disordered state (Chemat et al., 2017). During MAE, the moisture content of the sample matrix is primarily targeted for heating. As water evaporates, intracellular pressure rises, leading to the rupture of cell walls and enhancing the release of valuable compounds (Sosa-Hernández et al., 2018). In conventional extraction processes, heat is transferred from the heating medium to the inside of the cells, while solutes move in the opposite direction. In MAE, however, both heat and mass gradients work in the same direction, promoting the release of high-value compounds and reducing extraction time (Veggi et al., 2013).

As milk products are rich in MFGM, especially PLs, Costa-Junior et al. (2011) applied Supercritical Fluid Extraction (SCFE) for enrichment of phospholipids in whey buttermilk. Before SCFE, whey buttermilk was concentrated by ultrafiltration (10X) and subsequently diafiltered (5X) (10 kDa molecular mass cut-off membrane) at 25°C, and the final retentate was spray-dried. The whey buttermilk powder was submitted to supercritical extraction (350 bar, 50°C) using carbon dioxide. The membrane filtration removed most of the lactose and ash from the whey buttermilk, and the supercritical extraction extracted exclusively non-polar lipids. The final powder contained 73% protein and 21% lipids, of which 61% were phospholipids.

In a supercritical fluid extraction (SCFE) experiment on buttermilk, followed by two stages of microfiltration and diafiltration, the process used a flow rate of 20 g/min and a duration of 86.00 ± 0.06 minutes, consuming $1,560.0 \pm 116$ g of CO₂. This method led to a reduction in total fat (nonpolar lipids) by 38 to 55% and achieved a five-fold concentration of phospholipids (Spence et al., 2009).

In another study, the phospholipid concentrate was obtained from buttermilk using ultrafiltration with a 50 kDa membrane, resulting in an 8.5-fold increase in phospholipids. Further enrichment through supercritical fluid extraction (SCFE) with ethanol as a co-solvent reached a phospholipid

content of $56.24 \pm 0.07\%$ on a dry matter basis (D.M) (Barry et al., 2017).

In the production of whey protein phospholipid concentrate (WPPC), a combined 5-stage sequential extraction process was employed using 70% ethanol at 70°C. This method resulted in a total lipid recovery of 40.7%, a total phospholipid (PL) recovery of 58.1%, and achieved a phospholipid content of 45.8% (Price et al., 2018). The use of the switchable hydrophilicity solvent N, N-dimethylcyclohexylamine (CyNMe₂) demonstrated notable efficacy in recovering buttermilk and beta-serum. Recovery rates were significantly higher for buttermilk at $98.66 \pm 0.89\%$, while beta-serum recovery was notably lower at $7.67 \pm 0.51\%$. Additionally, there was a substantial nine-fold increase in phospholipid (PL) concentration, indicating the solvent's potential for enhancing lipid extraction efficiency in dairy processing (Rathnakumar et al., 2021).

Krishnegowda et al. (2026) have employed microwave-assisted extraction (MAE) for the extraction of phospholipids (PLs) from ghee residue under specific conditions: a power setting of 540 W, an extraction time of 60 seconds, and a solid-to-solvent (S:S) ratio of 10. The phospholipid yield (on a lipid basis) of 21.39% to 28.35% was reported under the optimized conditions, elucidating the efficiency of MAE in extracting phospholipids. Additionally, Krishnegowda et al. (2023 a and b) have reported the application of ultrasound-assisted extraction (UAE) and pulsed electric field (PEF) assisted extraction in improving the enrichment of phospholipids (PLs), raising their concentration from 4.98% to 20.57% to 30.17% and 11.85% to 18.13%, respectively, through a series of pre-treatments. The optimal conditions for UAE were identified as 80% power, a solvent temperature of 80°C, a solid-to-solvent (S:S) ratio of 15, and a sonication time of 4 minutes.

Conclusion

Thus, it can be concluded that advanced extraction technologies such as membrane, switchable solvents, microwave-assisted extraction and ultrasonication-assisted extraction allow enhanced recovery of bioactive compounds along with a reduction in required diffusion temperature, time, and quantity of diffusion solvents; thereby proving to be less energy-intensive for extraction of high-value compounds.

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