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Research Article

"Turning Waste Into Value: Extraction And Characterization Of Phospholipids From Ghee Residue"

Ms. Pande Shital Devidas[1*], Dr. S. C. Dhawale[2]

[1*]PhD Scholar, School of Pharmacy, Swami Ramanand Tirth Marathwada Univesity, Nanded 431606
Shitalpande08@gmail.com, Mob.No:7709988335
[2]Professor, School of Pharmacy, Swami Ramanand Tirth Marathwada Univesity,Nanded 431606

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ABSTRACT

This study presents a comprehensive approach for the extraction and characterization of phospholipids from ghee residue, emphasizing the conversion of waste material into valuable compounds. The extraction process involved successive washing with acetone and extraction using heated alcohol, followed by purification. Fractionation using silica-gel-G thin-layer chromatography facilitated the identification of distinct phospholipid fractions. Physical evaluations, including color, nature, melting point, and solubility, revealed the lipophilic nature of the phospholipids and their compatibility with organic solvents. TLC analysis yielded an RF value of 0.82, indicative of moderate mobility and separation efficiency. Molecular characterization via Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy provided detailed insights into the molecular structure, identifying characteristic functional groups such as alkyl, carbonyl, halogen, oxygen, vinylic, and aromatic groups. Notably, FTIR and NMR analyses unveiled deviations in frequencies, suggesting variations in chemical environments within the phospholipid molecules. Overall, this study offers a systematic approach for extracting and characterizing phospholipids from ghee residue, highlighting their potential applications in diverse research and industrial domains.

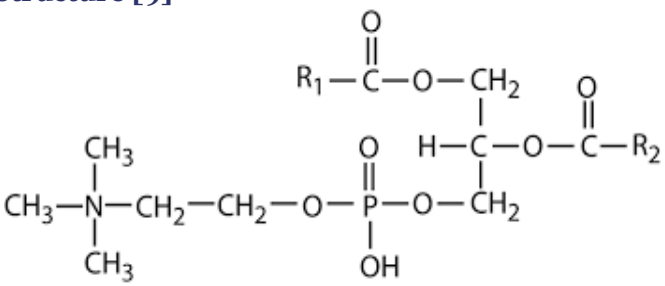
Keywords: Extraction, characterization, phospholipids, ghee residue, waste valorization, sustainability, analytical techniques, applications.

Introduction:

In recent years, the concept of valorizing waste materials to extract valuable compounds has gained considerable attention due to its economic and environmental benefits. Ghee, a clarified butter commonly used in South Asian cuisine, generates substantial residue during its production process. [1,2] This residue, often considered a waste byproduct, contains various lipid components, including phospholipids, which possess significant functional and nutritional properties. Phospholipids are essential constituents of biological membranes and play crucial roles in various physiological processes, making them valuable for pharmaceutical, food, and cosmetic industries. [3,4]

This study focuses on the extraction and characterization of phospholipids from ghee residue, aiming to harness these compounds from an underutilized source. [5,6] The extraction process involves multiple steps, including washing with acetone to remove impurities and extraction using heated alcohol to isolate phospholipids. Subsequent purification steps ensure the quality and purity of the extracted phospholipids. [7] Fractionation techniques, such as thin-layer chromatography, enable the separation of different phospholipid fractions, providing insights into their composition and properties. [8]

Phosphotidylcholine Structure [9]



The characterization of extracted phospholipids is essential to understand their molecular structure and functional attributes. Various analytical techniques, including Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) spectroscopy, and chromatographic analyses, are employed to elucidate the chemical composition and structural features of the phospholipids. [10,11]Through these analyses, information regarding the presence of specific functional groups and molecular interactions within the phospholipid molecules is obtained, facilitating their identification and characterization.

The findings of this study hold significant implications for both academic and industrial sectors. By valorizing ghee residue and extracting phospholipids, this research contributes to the sustainable utilization of waste materials and the development of value-added products. Moreover, the characterization of extracted phospholipids provides valuable insights into their potential applications in pharmaceutical formulations, functional foods, and cosmetic products. Overall, this study represents a step towards the efficient utilization of agricultural byproducts and the promotion of circular economy principles in the lipid industry.[12,13]

Materials and method:

Extraction of Phospholipid by Rose-Gottleib method

The ghee-residue was washed with three times its volume of cooled acetone (4'-5°C) three times, and the washings were discarded. The remaining substance was combined with an equivalent amount of ethyl alcohol at the ambient temperature, heated to 50-60°C, and then cooled back down to the ambient temperature. The lipids were extracted using a combination of ethyl ether and petroleum ether in equal proportions, following the Rose-Gottleib method (lSI, 1961). The method of extracting using alcohol, solvent ether, and petroleum ether was repeated three times, and the resulting extract was dried using a vacuum.The ghee-residue was subjected to a washing process using acetone, following the same approach as in part A. Afterwards, the phospholipids were extracted using heated alcohol, and this procedure of extracting with alcohol was done three times. The consolidated extract was subsequently dehydrated using a vacuum. The unrefined phospholipids acquired from techniques A and B underwent additional purification using the following procedure: The dehydrated lipids were dissolved in a minimal amount of ethyl ether, and the phospholipids were separated by precipitation using acetone at a temperature of 5°C. The process of precipitating with acetone was done six times. The phosphorus concentration was used to test the quality of the produced phospholipids.[14-18]

Fig:1 Extraction of Phospholipid by Rose-Gottleib method

The phospholipids obtained through methods A and B were subjected to fractionation on silica-gel-G thin-layer chromatography (E. Merck, according to Stahl) to identify the different fractions of phospholipids present.[19]

Evaluation

Physical Evaluation

Color: The color of the isolated phospholipids was visually assessed under standardized lighting conditions.[20]
Nature: The nature of the isolated phospholipids, including their texture, appearance was observed and recorded. [20]
Melting Point: The melting point of the isolated phospholipids was determined using a melting point apparatus according to established procedures. [20]
Solubility: The solubility of the isolated phospholipids was evaluated in various solvents of different polarities and temperatures, and the extent of solubility was quantified. [20]

TLC OF Phospholipids

To prepare the TLC solvent system for the analysis of phospholipids, approximately 160 mL of a mixture with the specified composition was formulated. This mixture was achieved by combining 45 mL of chloroform, 52.5 mL of ethanol, 10.5 mL of water, and 52.5 mL of triethylamine. The proportions were meticulously measured to maintain the desired ratio of chloroform, ethanol, water, and triethylamine at 30:35:7:35 (v/v/v/v). After thorough mixing, the resulting solvent system was ready for use as the mobile phase in TLC experiments, facilitating the separation and visualization of phospholipids based on their differential migration characteristics.[21,22]

Fourier Transform Infrared Spectroscopy (FTIR): The purified phospholipids were subjected to FTIR analysis to characterize their molecular structure and functional groups. FTIR spectroscopy provides information about the chemical bonds present in the sample by measuring the absorption of infrared radiation.[23]

UV-Visible Spectroscopy (UV): UV analysis was performed to assess the absorbance of the phospholipid samples in the ultraviolet-visible range of the electromagnetic spectrum. UV spectroscopy helps in determining the presence of conjugated double bonds or chromophores within the molecules, providing insights into the molecular structure and purity of the phospholipids.[24]

Nuclear Magnetic Resonance Spectroscopy (NMR):
NMR spectroscopy was utilized to elucidate the detailed molecular structure of the phospholipids. By measuring the nuclear magnetic properties of atoms within the sample, NMR provides information about the connectivity of atoms, stereochemistry, and molecular dynamics. In the context of phospholipids, NMR can reveal the fatty acid composition, headgroup structure, and overall conformation of the molecules.[25]

RESULTS AND DISCUSSION:

Molecular Weight: The molecular weight of the isolated phospholipids was determined to be 314.25 g/mol.
Color: The isolated phospholipids exhibited a pale yellow color.
Nature: The phospholipids were obtained in a solid state.
Melting Point: The melting point of the phospholipids was measured to be between 230°C and 231°C.
Solubility: The phospholipids showed solubility in chloroform and ethanol, indicating their lipophilic nature and compatibility with organic solvents commonly used in lipid analysis.
RF Value: The RF (retention factor) value for the isolated phospholipids in the TLC analysis was determined to be 0.82. This value indicates the degree of mobility of the compound relative to the solvent front and serves as a characteristic parameter for identification.

In the TLC experiment, the distance traveled by the compound was measured to be 1.9 units, while the distance traveled by the solvent front was recorded as 2.3 units. These values contribute to the calculation of the RF value, which is obtained by dividing the distance traveled by the compound by the distance traveled by the solvent front. In this case, an RF value of 0.82 suggests that the isolated phospholipids exhibit moderate mobility and separation efficiency under the given chromatographic conditions.

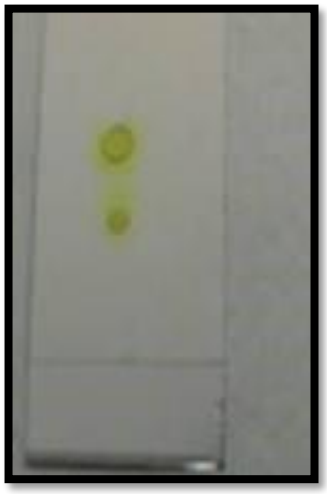


Fig 2: TLC of isolated phospholipids: RF value 0.82

Fourier Transform Infrared Spectroscopy (FTIR):

Fourier Transform Infrared Spectroscopy (FTIR) analysis of the purified phospholipids exhibited characteristic absorption bands corresponding to specific functional groups within the molecules. The observed frequencies were compared with reported values for identification and characterization purposes. The C–H stretching band of the long fatty acid chain displayed peaks at 2924.75 and 2859.89 cm^-1, slightly deviating from reported frequencies of 2918.3 and 2854.96 cm^-1. The carbonyl stretching band in the fatty acid ester appeared at 1740.20 cm^-1, while the expected frequency was 1728.22 cm^-1.

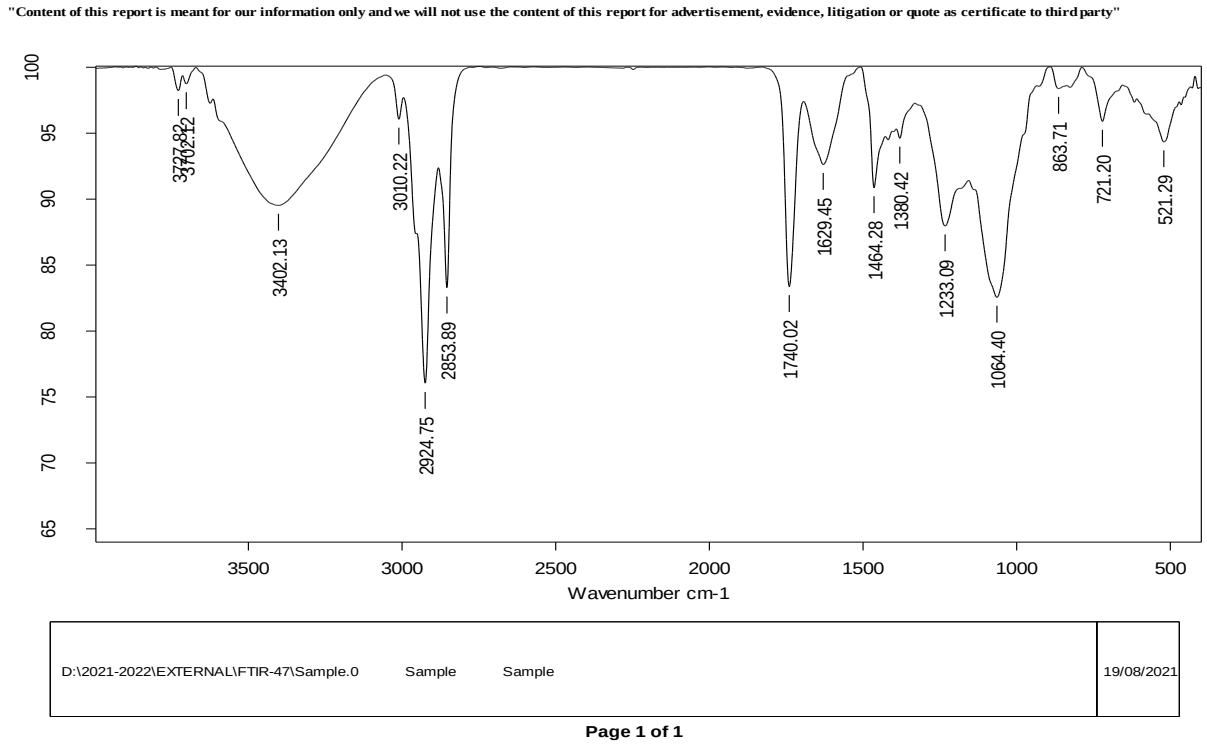


Fig 3: Fourier Transform Infrared Spectroscopy (FTIR) analysis of the purified phospholipids

Table 1: Fourier Transform Infrared Spectroscopy (FTIR) analysis

Sr.no	Characteristics Peaks	Reported frequency (cm-1)	Observed Frequency (cm-1)
1	C–H stretching band of long fatty acid chain	2918.3 and 2854.96	2924.75 and2859.89
2	Carbonyl stretching band in the fatty acid ester	1728.22	1740.20
3	C=O stretch α,β–unsaturated aldehydes, ketones	1710–1665	1629.45
4	P=O stretching band	1236.37	1233.09
5	P–O–C stretching band	1093.65	1064/40
6	N+(CH3)3 stretching	966.34	863.71
7	N-H/C-H/O-H Sreaching bond	3373-3442	3402.13

Additionally, the C=O stretch of α,β–unsaturated aldehydes and ketones showed an observed frequency of 1629.45 cm^-1 compared to a reported range of 1710–1665 cm^-1. The P=O stretching band and P–O–C stretching band were observed at 1233.09 cm^-1 and 1064/40 cm^-1, respectively, slightly differing from their reported frequencies. Furthermore, the N+(CH3)3 stretching exhibited an observed frequency of 863.71 cm^-1, while the reported frequency was 966.34 cm^-1. The N-H/C-H/O-H stretching bond appeared at 3402.13 cm^-1, consistent with the reported frequency range of 3373-3442 cm^-1. These deviations in frequencies may indicate variations in chemical environments or molecular interactions within the phospholipid molecules. FTIR analysis serves as a crucial analytical tool for qualitative and quantitative analysis, aiding in the identification and characterization of biomolecules in various research and industrial settings.

Nuclear Magnetic Resonance Spectroscopy (NMR):

Nuclear Magnetic Resonance (NMR) spectroscopy was employed to further elucidate the detailed molecular structure of the purified phospholipids.

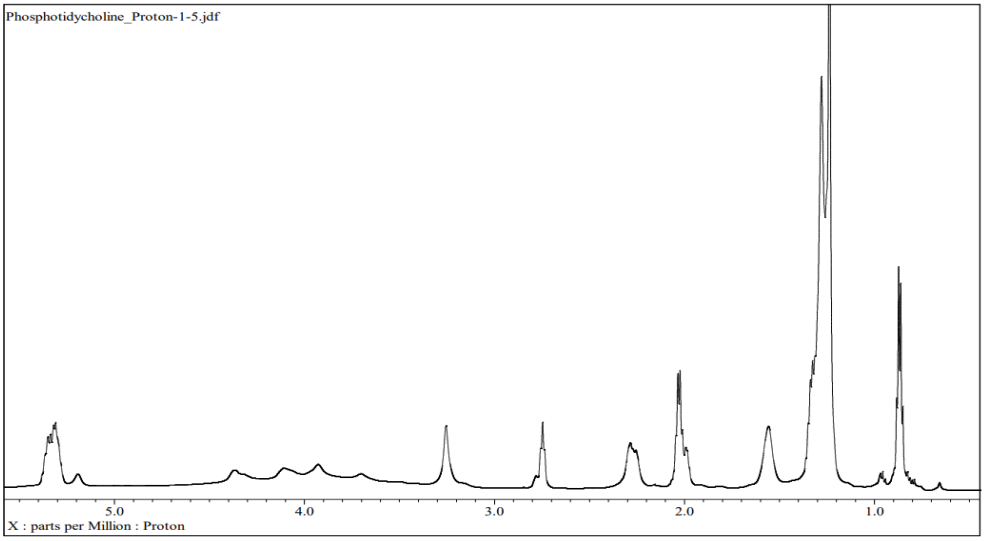


Fig 4: NMR: Phosphotidycholine-1H-e1

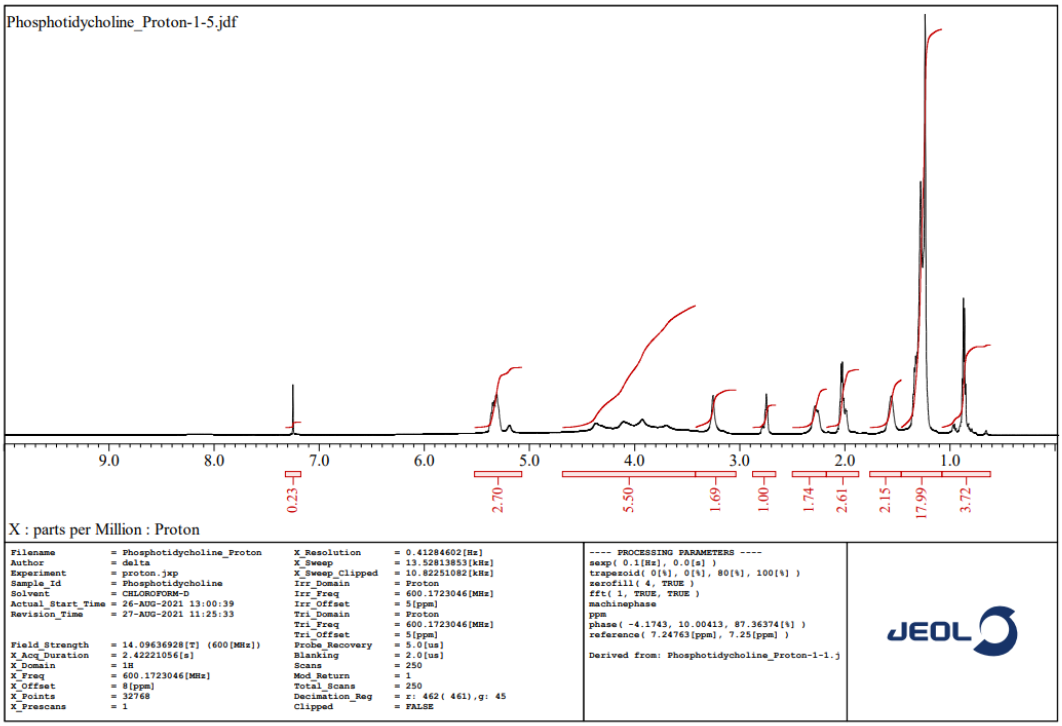


Fig 5: NMR: Phosphotidycholine-1H-f

In the proton (1H) NMR spectrum, characteristic peaks were observed, indicating the presence of various alkyl, carbonyl, halogen, oxygen, and vinylic functional groups within the phospholipid molecules. Peaks at 0.8 ppm,

1.30 ppm, and 1.6 ppm corresponded to methyl, methylene, and methine groups, respectively, while peaks at 2-2.3 ppm, 2.8 ppm, and 3.3 ppm indicated the presence of groups α to carbonyl, α to halogen, and α to oxygen, respectively. Additionally, a peak at 5.3 ppm suggested the presence of vinylic functionality, while a peak at 7.3 ppm indicated the presence of aromatic groups. In the carbon-13 (13C) NMR spectrum, peaks at 127-128 ppm and 129-130 ppm were assigned to nitriles and alkenes, respectively, while peaks at 173.11-173.62 ppm indicated the presence of carbonyl functionalities including ester, amide, and carboxylic acid groups.

Table 2: Nuclear Magnetic Resonance Spectroscopy

PEAK	Characteristic Peak
0.8	R-CH3 alkyl (methyl)
1.30	R-CH2-R alkyl (methylene)
1.6	R3C H alkyl (methine)
2-2.3	O R-C-CH3 α to carbonyl (C is next to C=O)
2.8	R-CH2-X α to halogen (C is attached to Cl, Br, I,P)
3.3	RO-CH3 α to oxygen (C is attached to O)
5.3	H R2C=CR vinylic (H is attached to alkene C)
7.3	Ar H aromatic (H is on phenyl ring)

C 13 NMR

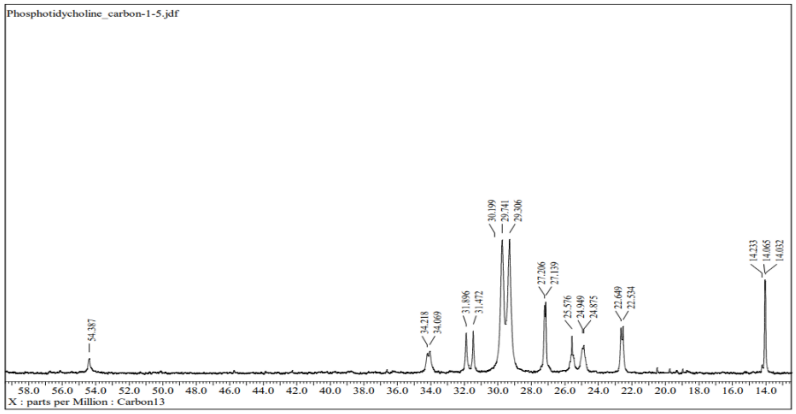


Fig 6: C 13 NMR: Phosphotidycholine-13C-e1

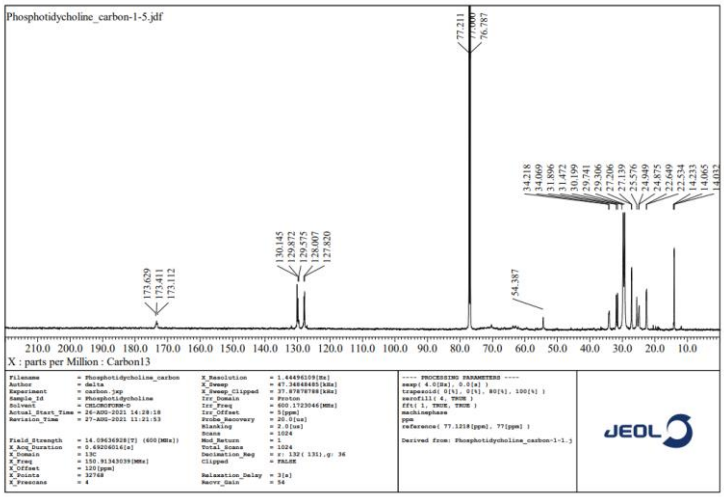


Fig 7: C 13 NMR: Phosphotidycholine-13C-f

Table 3: C 13 NMR

FREQUENCY	CHARACTERISTIC PEAK
127-128	Nitriles
129-130	Alkenes
173.11-173.62	Carbonyl: Ester Amide Carboxylic Acid

These characteristic peaks and their corresponding chemical shifts provide valuable information regarding the molecular structure and composition of the phospholipids, facilitating their identification and characterization in various research and industrial applications. NMR spectroscopy serves as a powerful analytical tool for elucidating the structural features of biomolecules and organic compounds, contributing to our understanding of their chemical properties and biological functions.

HRLCMS

High-Resolution Liquid Chromatography Mass Spectrometry (HRLCMS) analysis of the purified phospholipids revealed several peaks in the mass spectrum, indicative of different phospholipid species present in the sample. Each peak in the spectrum corresponds to a specific m/z value (mass-to-charge ratio), providing information about the molecular weight of the phospholipids. The calculated m/z values were compared with theoretical values to determine the accuracy of the measurements, with differences expressed in parts per million (ppm).

Peak analysis revealed prominent signals at m/z 312.361, 496.3369, 497.3404, 498.3427, 518.3189, 520.3363, 522.3519, 534.2926, and 991.6659, among others. These peaks represent different phospholipid ions detected in the sample, with varying abundances and charge states. Notably, the most intense peak observed at m/z 312.361 corresponds to the protonated molecular ion [M+H] + of a phospholipid species, indicating its prevalence in the sample.

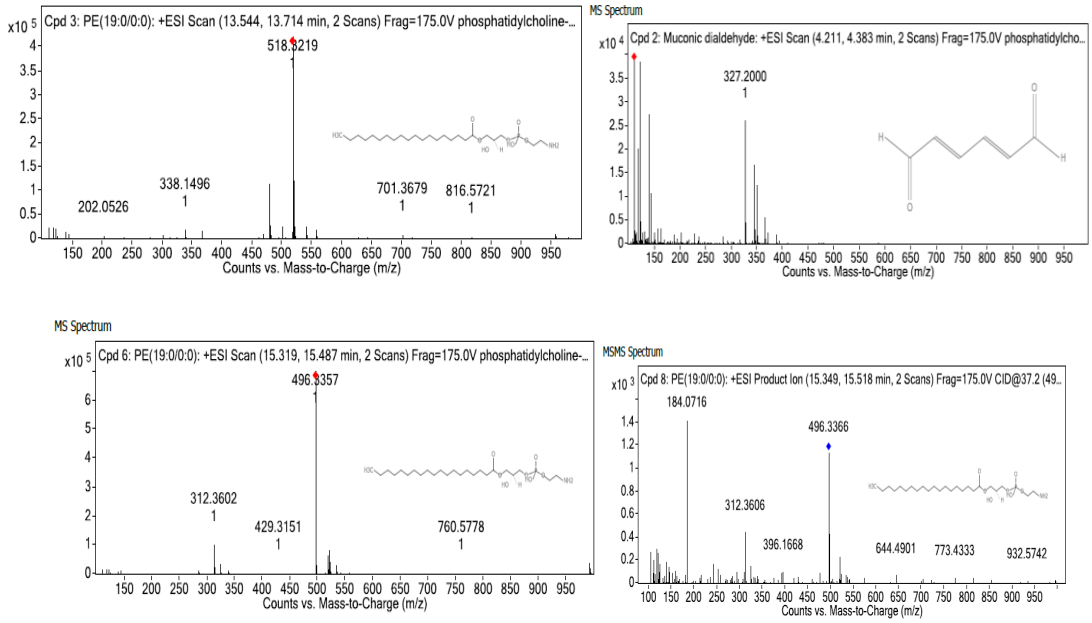


Fig 8: HRLCMS of the purified phospholipids

Comparison between the calculated and theoretical m/z values revealed small differences, ranging from 5.4 to 6.08 ppm, suggesting high accuracy in mass measurement. This high level of accuracy enhances the reliability of the HRLCMS analysis and ensures the precise identification of phospholipid species present in the sample.

Further analysis of the identified phospholipid ions revealed their corresponding molecular formulas, providing insights into their chemical compositions. For instance, the phospholipid species detected at m/z 312.361 was identified as C24H50NO7P, representing a phospholipid molecule with 24 carbon atoms, 50 hydrogen atoms, one nitrogen atom, seven oxygen atoms, and one phosphorus atom.

Table 4: HRLCMS of the purified phospholipids

MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
312.361			1	91705.71		
324.2879			1	27767.87		
496.3369	496.3398	5.82	1	635670.69	C24 H50 N O7 P	(M+H)+
497.3404	497.3431	5.49	1	164092.23	C24 H50 N O7 P	(M+H)+
498.3427	498.3457	6.08	1	29746.65	C24 H50 N O7 P	(M+H)+
518.3189			1	59113.88		
520.3363			1	64997.09		
522.3519			1	48056.6		
534.2926			1	29416.74		
991.6659			1	26715.18		

The abundance of each phospholipid species, expressed in arbitrary units, varied among the detected ions, with some species exhibiting higher abundances compared to others. This variation in abundance reflects the heterogeneous nature of the phospholipid mixture and highlights the complexity of the sample composition.
Overall, the HRLCMS analysis provides valuable information about the molecular composition and diversity of phospholipids present in the purified sample. The accurate determination of m/z values, coupled with molecular formula assignment, enhances our understanding of the phospholipid profile and paves the way for further investigations into their structural properties and biological functions. This detailed characterization of phospholipids is essential for elucidating their roles in biological systems and exploring their potential applications in various fields, including pharmaceuticals, nutrition, and biotechnology.

Conclusion:

In conclusion, this study demonstrates a systematic approach for the extraction and characterization of phospholipids from ghee residue, highlighting the potential of waste valorization in generating value-added products. Through successive washing and extraction processes, phospholipids were effectively isolated from the ghee residue, indicating the feasibility of utilizing this byproduct as a source of valuable lipids. Fractionation techniques further enabled the separation of distinct phospholipid fractions, providing valuable insights into their composition and properties.The comprehensive characterization of extracted phospholipids using analytical techniques such as FTIR, NMR spectroscopy, and chromatography revealed detailed information about their molecular structure and functional groups. Deviations observed in frequency values during FTIR analysis suggest variations in chemical environments within the phospholipid molecules, further emphasizing the need for thorough characterization to understand their properties. The findings of this study have significant implications for various industries, including pharmaceuticals, food, and cosmetics. The extracted phospholipids hold promise for applications in drug delivery systems, nutritional supplements, and skincare formulations due to their biocompatibility and functional properties. Moreover, the utilization of ghee residue as a renewable source of phospholipids contributes to the sustainable utilization of agricultural byproducts and promotes circular economy principles. Future research endeavors could focus on optimizing extraction processes to improve yield and purity of phospholipids, as well as exploring novel applications in emerging fields such as nanotechnology and biomedicine. Additionally, further investigations into the functional properties and biological activities of extracted phospholipids would provide deeper insights into their potential benefits and applications. Overall, this study underscores the importance of waste valorization strategies in creating value from underutilized resources and highlights the potential of phospholipids derived from ghee residue as versatile and valuable compounds with diverse applications in various industries. By bridging the gap between waste management and resource utilization, this research contributes to the advancement of sustainable practices and innovation in the lipid industry.

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