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Comparative Phospholipid Profiling of Human Milk and Infant Formulas Using LC-MS/MS and a Multi-Parameter Similarity Evaluation Model

Huiquan Zhu[1,2], Marie-Laure Fauconnier[2], Hong Zhang[3], Xuebing Xu[3], Ruihua Guo[3], Shuwen Zhang[1], Xiaodan Wang[1], Jiaping Lv[1*], Xiaoyang Pang[1*], Yunna Wang[1*]

[1] Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, China

[2] Laboratory of Chemistry of Natural Molecules, Gembloux Agro-bio Tech, University of Liege, Gembloux, 5030, Belgium

[3] Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd., Shanghai 200137, China

* Correspondence:

Corresponding Author: Jiaping Lv, Xiaoyang Pang, Yunna Wang

E-mail: lvjiapingcaas@126.com, pangxiaoyang@163.com, wang_yn92@163.com

Abstract:

Human milk phospholipid (HMPL) plays an indispensable role in infant growth, while there are few studies focused on the comprehensive phospholipid composition between human milk and infant formula. In this study, LC-MS/MS and a similarity evaluation model were used to compare phospholipid composition from human milk and infant formula. The results showed that the number of phospholipid molecules detected in HM (137) was higher than that in CIFs (86) and GIFs (77). Furthermore, the similarity evaluation showed that although the average comprehensive similarity score for CIFs (65.68) was higher than that for GIFs (53.62), their phospholipid compositions differed significantly from HMPLs. Therefore, these results not only deepen our understanding of

HM phospholipids but also provide a new scientific basis for the development of infant formulas.

Keywords: phospholipid, human milk, infant formulas, evaluation model

1. Introduction

Lipids are the second-largest nutrient in human milk (HM) and can provide approximately 50 % of infants' energy requirements. They are mainly composed of triglycerides, phospholipids (PL), gangliosides, and other lipids. Human milk phospholipids (HMPLs) are polar lipids, accounting for 0.2 – 2 % of total lipids. Most HMPLs are located at the milk fat globular membrane, accounting for about 65 %, while the remaining HMPLs exist in the aqueous phase and are associated with the protein/membrane segment material in solution (Cossignani et al., 2019; Yang et al., 2022). HMPLs are mainly composed of sphingomyelin (SM, 38.06 – 47.62 %) and glycerophospholipids, predominantly containing phosphatidylcholine (PC, 29.61 – 33.83 %), phosphatidylethanolamine (PE, 10.54 – 24.46 %), phosphatidylinositol (PI, 0.43 – 4.80%), and phosphatidylserine (PS, 4.00 %) (Fong et al., 2020; Song et al., 2021; Zhu et al., 2023). HMPLs provide choline and polyunsaturated fatty acids during infant growth, such as DHA (C22:6n3) and EPA (C20:5n3), supporting infants' neurodevelopment and visual function. Moreover, HMPLs are involved in regulating cell integrity and mobility in comparison with the triglycerides (Sun et al., 2018; Wang et al., 2024).

Due to the nutritional needs of infants, research on HMPLs has been increased recently. The studies mainly focus on factors that influence HMPL concentration, including lactation period, sampling region, daily diet, and physical state of the voluntary mothers. For the lactation periods, most researchers reported that the total HMPL concentration decreased with the extension of lactation, from the colostrum (204.44 – 330.90 mg/L) to mature milk (170.00 – 219.50 mg/L) (Ma et al., 2017;

Zhao et al., 2021). However, Zou et al. (2017) reported the highest total HMPLs concentration was observed in human transitional milk (Zou et al., 2017). The phospholipid classes concentration showed the similar variation with the total HMPL concentration during whole lactation periods, while Ma et al. (2017) found an increasing of SM concentration (38.30 to 65.40 mg/L) (Ma et al., 2017). The sampled region was another important affecting factor, the total HMPL concentration was 36.94 mg/100 mL in Ireland, significantly higher than that in France (25.03 mg/100 mL) and Singapore (20.78 – 24.24 mg/100 mL) (Ingvordsen Lindahl et al., 2019; Thakkar et al., 2013). Moreover, Zhu et al. (2023) reported that the highest total HMPL concentration was detected in Zhengzhou among 8 different Chinese sampling regions (Zhu et al., 2023). Due to the different detection methods used in the experiments, the phospholipid units reported in different literatures were inconsistent. For more detailed information, please referred to Table S1. This different phenomenon might be caused by the diversification of dietary habits; Europeans consumed dairy products and palm oil, and people in China preferred soybean oil for cooking. Furthermore, certain studies found that PC 36:2, PE 36:2, and SM 40:1 were the predominant phospholipid molecules in HMPLs, accounting for the 19.26 – 33.00 % of total PC, 33.97 – 59.45 % of total PE, and 17.22 – 39.80 % of total SM, respectively (Wei et al., 2022; Zhu et al., 2023).

Infant formulas, mainly produced by cow milk, goat milk or their products, are the ideal substitutes for breast milk, playing an irreplaceable role in the supply shortage of human milk (Bakshi et al., 2023). It has been reported that the total phospholipid concentration is 25.11 - 58.07 mg/100 mL in infant formulas (Claumarchirant et al., 2016). Furthermore, the concentration of PC, PE, and SM is 1.5 – 75.0 mg/100g, 1.8 – 84.0 mg/100g, and 0.0 – 82.0 mg/100g, respectively (Cilla et al., 2016).

A recent study found that there were fewer kinds of phospholipid molecules in infant formulas (159) in comparison with HMPLs (231), and the SM concentration (35.84 ± 15.56 mg/L) in infant formulas was significantly lower than that of HMPLs (45.53 ± 16.04 mg/L) (Liu et al., 2023). Recently, to meet the nutritional needs of infants, the natural phospholipids are used in infant formulas, such as plant-derived phospholipids (soybean phospholipid (SBP) and sunflower phospholipid), egg phospholipid, and cow milk phospholipid (milk fat globule membrane). Due to the high yield and low production cost price, SBP, enriched in PC (>60 % of total phospholipids), is widely chosen and used in infant formulas for phospholipid supplement (Ma et al., 2024). However, SBP often are added in a single type, and there were the significant differences in phospholipid classes and molecules between HMPLs and SBP (Zhu, Fauconnier, et al., 2024), and there currently is no specific standard for the phospholipid addition of infant formula.

To improve the infant formula quality, the similarity model is used to calculate the similarity between human milk and its substitutes. For HMPLs, however, there is only one individual three-dimensional similarity evaluation model established by Bray-Curtis similarity index (Zhu, Fauconnier, et al., 2024). Moreover, it still has shortcomings. There are significant individual variations in human milk components. Suppose the average value is used solely to evaluate the similarity between human milk and its substitutes. In that case, dynamic differences in human milk will be ignored, which are influenced by daily diet, sociological factors, lactation period, and maternal physical condition. Hence, it is essential to find a new similarity evaluation model to evaluate the HMPL replacement from multiple perspectives. Although Wang et al. (2010) and Zou et al. (2013) and proposed a similarity model, which was based on the deduction principle and

dynamic data of human milk fatty acids and triglycerides, it had some shortcomings and did not include HMPLs (Wang et al., 2010; Zou et al., 2013). Therefore, it is necessary to improve this evaluation model; TOPSIS (technique for order preference by similarity to ideal solution) is a comprehensive evaluation method, which is commonly used in multi-index decision-making. Its basic principle is to find an "ideal best solution" and an "ideal worst solution" in the multi-dimensional index space, and rank the alternatives based on their distances from these two ideal points. In this study, the deduction principle was combined with the TOPSIS method for optimization, and then used it to evaluate the phospholipid composition similarity of different infant formulas from multiple perspectives, including phospholipid concentration, classes, molecules, and fatty acids (PLFAs). These results will provide new scientific data for the mimicking of human milk lipid and offer new insights for the further development and utilization of infant formulas.

2. Materials and methods

2.1 Materials

Chromatographic-grade methanol (MeOH), chloroform ($CHCl_3$), isopropyl alcohol, acetonitrile, and n-hexane were purchased from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Anhydrous sodium chloride and potassium hydroxide were obtained from Sinopsin Chemical Reagent Co., LTD. (Beijing, China). A mixture of 37 FA methyl ester was provided by Ampere Laboratory Technology Co., LTD. (Shanghai, China). Phospholipid standards, including 1,2-dimyistoryl-sn-glycero-3-phosphocholine (>99%), sphingomyelin (>99%), 3-sn-Phosphatidyl-L-serine (bovine, >97%), 1,2-dimyistoryl-sn-glycero-3-phosphoethanolamine (>97%), l-alpha-phosphatidylinositol (>98%), 1-myristoyl-2-hydroxy-sn-glycero-3-phosphate (>99%), and 1-lauroyl-2-hydroxy-sn-glycero-3-

phosphocholine (>99%), were purchased from Avanti Polar Lipids (Birmingham, AL, U.S.A.).

2.2 Milk samples

Human mature milk samples (n=10, obtained from Beijing, China) were provided by the China Human Milk Project (CHMP) and collected by trained personnel. This project was registered in Clinical Trials.gov., which recruited a total of 1800 volunteers from multiple sampling regions in China (ID number: NCT03675204). After breast cleansing, 60 mL of milk was obtained from each voluntary donor using an electric breast pump, aliquoted, flash-frozen in liquid nitrogen, and stored at -80 °C. Sampling was conducted between 9:00 a.m. and 11:00 a.m. All participants provided informed consent and met the inclusion criteria: healthy lifestyle, no smoking or alcohol consumption, and absence of infectious, cardiac, metabolic, or chronic diseases. Donors were 20 – 35 years old and had delivered at full term. All voluntary mothers clarified they were not directly involved in this project, and a third party provided the samples. The project handled all ethical approvals.

Infant formulas (n = 30), all Phase 1 formula (for infants aged 0-6 months), were divided into two groups based on the primary milk source: cow milk-based infant formulas (CIFs, n = 18) and goat milk-based infant formulas (GIFs, n = 12). Four groups were defined based on phospholipid supplementation: CIFG1 (CIFs with SBP, n = 10), CIFG2 (CIFs without SBP, n = 8), GIFG1 (GIFs with SBP, n = 6), and GIFG2 (GIFs without SBP, n = 6). Products were purchased from a local supermarket in Beijing, China.

2.3 Extraction of milk fat

Milk samples (1 mL) were mixed with 12 mL of $CHCl_3$/MeOH (2:1, v/v), shaken vigorously for 10

min, followed by the addition of NaCl solution and vortexing (Vortex Genie 2, SI-0246, Scientific Industries, United States) for 2 min. After centrifugation (3500 rpm, 15 min, Sigma 3K15, Sigma Laborzentrifugen, Germany), the supernatant was collected and dried under nitrogen to obtain lipids for further analysis (Termovap Sample Concentrator, ND400, Hangzhou Ruicheng Instrument Co.,Ltd, China). To dissolve the infant formulas, 1.3 g of infant formula was weighed in a tube, and then 9 mL of ultra-pure water (<18.2 MΩ·cm) was added. The mixed solution was vortexed with a dissolving temperature of 60 ± 5℃. The fat extraction process for infant formulas was the same as for HM. (Hormozi Jangi & Khoobi, 2024).

2.4 Detection and quantification of phospholipid

Five milligrams of the lipid samples were accurately weighed and dissolved in 1 mL of $CHCl_3$/MeOH (1/2, v/v). After that, samples were diluted 50 times with MeOH containing 10 mmol/L ammonium formate and 0.10 % formic acid, and then they were transferred to liquid chromatography detection (LC, Waters Corporation, Milford, United States). PC, PE, SM, PI, ceramide (Cer), cerebroside (Cb), lysophosphatidylethanolamine (LPE) and lysophosphatidylcholine (LPC) were analyzed in positive ion mode, with LPC12:0 as the quality control. PS, lysophosphatidyl serine (LPS), and lysophosphatidylic acid (LPI) were detected in negative ion mode using lysophosphatidic acid 14:0 (LPA14:0) for quality control.

In positive-ion mode, mobile phases A and B were consisted of isopropyl alcohol/acetonitrile (90/10, v/v) and acetonitrile/water (70/30, v/v), respectively, both containing 10 mM ammonium formate and 0.1 % formic acid. In negative-ion mode, mobile phases C and D were consisted of methanol/water (90/10, v/v) and isopropanol/acetonitrile (90/10, v/v), respectively, both containing

0.1 % ammonium hydroxide ($NH_4OH•H_2O$). The LC separation was conducted through the BEH C18 column (1.7 μm, ID 2.1 mm × 100 mm, Waters, USA) and a short C18 column (5 μm, ID 2.1 mm × 20 mm, r-0121-c185, Higgins Analytical, Southborough, MA), which were used for positive and negative ionization modes, respectively. Mass spectrometry was performed using an API 4500 QTRAP Mass Spectrometer (SCIEX, USA). Specific mass spectrum conditions were described in a previous study (Zhu, Fauconnier, et al., 2024).

Major phospholipid classes (PC, PE, PI, PS, and SM) in HM and infant formulas were quantified by external standard method (Jiang et al., 2022). Preparing standard solutions of different concentrations of phospholipid standards and plotting standard curves, the concentrations of phospholipid classes were calculated based on peak areas. The limit of detection (LOD) and limit of quantitation (LOQ) for each phospholipid class were determined based on the LC-MS/MS sensitivity, with LOD and LOQ being 3 and 10 times of the system noise level, respectively. Additionally, the intra-day and inter-day repeatability was measured by injecting each phospholipid standard sample 3 times and 3 days, respectively, and their repeatability were expressed as relative standard deviation (RSD).

2.5 Purification of phospholipid fatty acids

PLFAs was purified using a gradient elution method. Lipid sample (50 mg) was loaded onto a silica gel SPE column (CNWBOND Si, 1 g, 6 mL). A series of n-hexane/ether eluents in different proportions (50/1, v/v (5 mL), 6/1,v/v (3 mL), 1/1,v/v (1 mL)), pure methanol (6 mL), and mixed solution ($CHCl_3$/MeOH/H_2O, 3/5/2, v/v/v/, 3 mL) were then used to obtain phospholipids. After nitrogen drying (Termovap Sample Concentrator, ND400, Hangzhou Ruicheng Instrument Co.,Ltd, China), 14 % boron trifluoride methanol solution (300 μL) was added and heated at 100 °C for 90

min. After cooling, 600 μL n-hexane was added, and the mixture was dried over anhydrous sodium sulfate. The supernatant was filtered (0.22 μm) and analyzed using the gas chromatography with flam ionization detection (GC8890, Agilent 8890B, United States) (Heydari et al., 2019; Khoobi et al., 2019; Si et al., 2023; Zhu, Wang, et al., 2024) .

2.6 Establishment of similarity evaluation model for human milk phospholipid

Compared to the original model (based on deduction principle) (Wang et al., 2010; Zou et al., 2013), the offset rate of modified model was fixed according to the TOPSIS method, which conducted a comprehensive evaluation by assessing the degree of each scheme's proximity to the ideal solution, and it could evaluate the attributes of items from multiple perspectives. The workflow and principles of the evaluation model are shown in Figure S1. To more directly demonstrate the improvement of the evaluation model, we used an example to substantiate it. Assuming one similarity index for two different infant formulas was detected at 9 and 21, respectively, with a corresponding HMPL range of 10 - 20. Their corresponding offset rates (Ci), obtained from the original model, were 0.1 and 0.05, respectively, while these two offset rates should be consistent. Therefore, we assigned fixed values to ensure the accuracy of the offset rate and to further guarantee the model's precision (Figure S1). A total of 39 similarity indexes were divided into four groups in individual evaluation model for HMPLs, including phospholipid concentration, phospholipid class distribution, PLFAs, and phospholipid molecules.

2.7 Statistical analysis.

Data analysis was conducted using SPSS 24.0 (SPSS, Chicago, IL, USA), with results expressed as mean ± standard deviation. Shapiro-Wilk test and Levene test were used to access the normality and uniformity of variance. Principal component analysis (PCA) and orthogonal partial least squares

discriminant analysis (OPLS-DA) were performed using SIMCA (14.1) to explore the PL molecular species difference between HM and infant formulas.

3. Results and discussion

3.1 Phospholipid fatty acids

A total of 34 kinds of PLFAs were detected across all samples. Among them, 33 PLFAs were identified in HM, the highest number observed, followed by GIFs (30) and CIFs (29) (Table 1 and Figure 1g). In HM, the most abundant PLFAs were palmitic acid (C16:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), and stearic acid (C18:0) (Figure 1). The palmitic acid had the highest content, accounting for 26.80 % of total PLFAs, followed by oleic acid (22.00 %), linoleic acid (17.09 %), and stearic acid (10.12 %). This result was consistent with the previous findings (Wei et al., 2022; Zhu, Fauconnier, et al., 2024). Unsaturated fatty acids are crucial for infant growth, the mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) in HM accounted for 33.60 % and 23.53 % of total PLFAs, respectively. For DHA and arachidonic acid, which were essential for infant brain development, their contents were 0.38 % and 0.65 % of total PLFAs in HM, respectively (Vázquez et al., 2024; Wang et al., 2021). These results aligned with previous reports (Zhu, Wang, et al., 2024).

Similar to the composition of HMPLs, the palmitic acid, oleic acid, linoleic acid, and stearic acid were the predominant PLFA species in both CIFs and GIFs, collectively accounting for over 70 % of total PLFAs. In CIFs, the average oleic acid content was 27.68 % of total PLFAs, which surpassed the proportion of palmitic acid (26.91 %). Conversely, in GIFs, palmitic acid was more abundant

(30.77 %) than oleic acid (22.46 %). Moreover, the average MUFA content in CIFs and GIFs was 35.00 % and 32.90 % of total PLFAs, respectively, closely resembling that of HM. However, the PUFA content in infant formulas was slightly lower than in HM, accounting for 20.46 % and 16.88 % of total PLFAs in CIFs and GIFs, respectively. Following the SBP addition, the average content of palmitic acid, oleic acid, linoleic acid, MUFA, and PUFA in CIFG1 and GIFG1 was comparable to those in CIFG2 and GIFG2. Regarding DHA, which was of particular interest, its content was 0.29 % and 0.26 % of total PLFAs in CIFs and GIFs, respectively, which were both lower than that of HMPL. DHA not only plays an essential role in the development of infant brain and vision, but also DHA supplementation can improve infant immunity and reduce the incidence of eczema in offspring. (Li et al., 2021).

3.2 Phospholipid classes

Subsequently, we conducted qualitative and quantitative analysis of phospholipid in HM and infant formulas. A total of 11 lipid classes were detected in all samples, including 4 glycerophospholipid species (PC, PE, PI, and PS), 4 lyso-phospholipid classes (LPC, LPE, LPI, and LPS), and 3 sphingolipid classes (SM, Cer, and Cb). In terms of relative content (Figure 2a), calculated by peak area, SM was the most abundant phospholipid class in HM, accounting for 40.96 % of total PLs, followed by PC (37.93 %), PE (14.68 %), PI (1.33 %), and PS (0.59 %). These findings were consistent with previous studies (Wei et al., 2022; Zhu, Fauconnier, et al., 2024). In contrast, PC was the dominant phospholipid class in infant formulas, representing 51.33 % and 52.82 % of total PLs in CIFs and GIFs, respectively, likely due to the naturally high PC content in animal milk. It was interesting that the PC content in CIFG1 and GIFG1 was higher than in CIFG2 and GIFG2, attributable to the addition of SBP. SM was the second most abundant phospholipid class in CIFs

and GIFs, lower than that in HM, accounting for 29.57 % and 33.48 % of total PLs, respectively. Studies have shown that PC and SM serve as primary choline sources essential for the development of infant brain and nervous system, with SM- and PC-fortified infant formula enhancing cognitive development in low-birth-weight infants (Garcia et al., 2013; Tanaka et al., 2013). Additionally, SM affected the gut microbiota; mice fed milk-derived SM showed higher abundances of Bacteroidaceae and Bifidobacterium compared to those fed egg-derived SM, which might help reduce the negative effects of a high-fat diet. (Yuan et al., 2024). The contents of lyso-phospholipids, Cer, and Cb were lower both in HM and infant formulas. Among lyso-phospholipids classes, the LPC content was the highest, accounting for 1.29 %, 0.60 %, and 1.01 % of total PLs in the HM, CIFs, and GIFs, respectively. Notably, LPS was not detected in CIFs and GIFs.

Five major phospholipid classes (SM, PC, PE, PI, and PS) in HM and infant formulas were quantified using an external standard method (Figure 2b). The intra-day and inter-day repeatability was 0.31 – 4.50 % and 2.02 – 5.06 %, respectively. The correlation coefficient (R^2) ranged from 0.9932 to 0.9999, and the sample recovery rate ranged from 92.18 % to 105.06 %. The LOD and LOQ of different phospholipid standard were shown in Table S2. The average total concentration of HMPL was 19.53 mg/100mL, with SM being the most abundant (8.09 mg/100 mL), followed by PC (5.79 mg/100mL), PE (5.43 mg/100mL), PI (0.15 mg/100mL), and PS (0.06 mg/100mL). In contrast, the average total concentration of phospholipid in infant formulas was significantly lower in comparison with that of HMPLs, measuring only 10.88 mg/100mL and 4.74 mg/100mL in CIFs and GIFs, respectively. Although researchers recognized the importance of HMPLs, there are currently no clear standards for the amount of phospholipid additive in infant formulas, resulting in

variability and uncertainty in phospholipid content during production. It might also be due to the loss of phospholipids during the production and testing processes. For the individual phospholipid classes, PC was the most abundant in infant formulas, with concentrations of 4.35 mg/100 mL and 2.12 mg/100 mL in CIFs and GIFs, respectively, concurring with prior findings (Claumarchirant et al., 2016). However, the SM concentrations, with 2.29 mg/100mL and 1.20 mg/100mL in CIFs and GIFs, respectively, were lower than those in previous research (Claumarchirant et al., 2016; Liu et al., 2023). A study on foreign infant formulas reported the total phospholipid concentrations ranged from 25.11 to 58.07 mg/100 mL, with PE being the most abundant class, exceeding the values observed in this study (Claumarchirant et al., 2016). These discrepancies might be attributed to variation in formulation, processing techniques, and analytical methods. This finding underscored the importance of optimizing total phospholipid concentrations in future infant formula production.

3.3 Phospholipid molecules

A total of 137, 86, and 77 kinds of phospholipid molecules were detected in HM, CIFs, and GIFs, respectively, of which the number of PC, PE, and SM molecules was more abundant (Table S2). After that, the area normalization method was employed to compare and analyze the distribution of phospholipid molecules across all samples (Figure 3b).

3.3.1 Glycerophospholipids

PC36:2, PC36:1, PC34:1, and PC34:2 were the predominant PC molecules in HM, and the PC36:2 content was the most abundant, accounting for 23.02 % of total PC. In contrast, PC34:1, PC36:4, PC34:2, and PC36:2 were more prevalent than other PC molecular species in infant formulas. The PC34:1 content was the highest in infant formulas, with average content of 18.99 % and 18.58 % of total PC in CIFs and GIFs, respectively. This result is in line with findings in raw cow milk and goat

milk (Wei et al., 2022). Notably, the PC36:4 content in CIFG1 (15.06 % of total PC) and GIFG1 (21.07 % of total PC) was significantly higher than that in CIFG2 (2.11 %) and GIFG2 (3.04 %). This phenomenon might be caused by the addition of SBP, of which PC36:4 was the predominant PC molecule.

PE36:2 was the most abundant molecule across all samples, accounting for 47.21 %, 35.73 %, and 35.14 % of total PE in HM, CIFs, and GIFs, respectively. Similar to PC, the contents of PE36:4 and PE34:2 increased significantly in SBP-supplemented groups (CIFG1 and GIFG1), this results was consistent with SBP, of which PE36:4 and PE34:2 content accounted for over 60 % of total PE (Sun et al., 2018; Zhu, Fauconnier, et al., 2024). Although PI and PS were present in lower content, PS had been shown to delay cognitive decline related to memory and learning impairment and PI contributed to cholesterol reduction (Richter et al., 2013). A total of 7 PI and 6 PS molecules were detected in HMPLs, and PI34:1, PI36:2, and PS36:0 were the predominant, accounting for 18.8 % of total PI, 26.18 % of total PI, and 33.30 % of total PS, respectively, which was in accordance with previous findings (Zhu, Fauconnier, et al., 2024). Similar molecular profiles of PI and PS were identified in infant formulas, with PI34:1 being the most prevalent, representing 40.77 % of total PI in CIFs and 31.02 % in GIFs, respectively. Furthermore, PS34:1 content was the highest, accounting for 45.63 % and 22.27 % of total PS in CIFs and GIFs, respectively.

3.3.2 Sphingolipid

SM is able to participate in myelin sheath formation and brain function (Schneider et al., 2019), and SM supplementation has been shown to reduce plasma insulin levels and contribute to lipid metabolism in both serum and the liver (Yuan et al., 2024). Through the area normalization method,

we found that SM40:1, SM38:1, SM34:1, and SM42:1 were the predominant SM molecules in HMPLs. The SM40:1 content was the highest, accounting for 25.85 % of total SM, followed by SM38:1 (12.32 %), SM34:1 (8.38 %), and SM42:1 (8.52 %). These findings are consistent with previous studies (Fong et al., 2020; Ingvordsen Lindahl et al., 2019; Li et al., 2020). The number of SM molecules in infant formulas was less than in HM. In these formulas, the contents of SM34:1, SM38:0, SM38:1, and SM40:1 were higher than those of other molecules. Among them, SM38:0 and SM34:1 were the predominant molecules in CIFs (15.58 % of total SM) and GIFs (20.70 % of total SM), respectively.

There are relatively few reports on the presence of Cer in milk, which plays a vital role in maintaining the skin's osmotic barrier (Meckfessel & Brandt, 2014). Nine Cer molecules were identified in HMPLs, with Cer24:1 being the most abundant (32.32 % of total Cer), concurring with previous findings (Zhu, Fauconnier, et al., 2024). In infant formulas, only four Cer molecules were detected; Cer24:0 and Cer16:0 were the predominant molecules in CIFs and GIFs, respectively, accounting for 33.34 % and 45.84 % of total Cer, respectively. For Cb, the content of Cb24:1, Cb22:0, and Cb24:0 was the highest in HM, CIFs, and GIFs, accounting for 33.45 %, 44.66 %, and 32.05 % of total Cb, respectively.

3.3.3 Lysophospholipids

Lysophospholipids play a functional role, such as facilitating faster delivery and absorption of PUFAs in humans body. (Yalagala et al., 2025). A total of 28 kinds of lysophospholipids species were detected across all samples, with LPC being the most diverse class (9 species identified). LPC16:0 was the predominant molecule both in HM and CIFs, taking up 31.11 % and 51.97 % of

total LPC, respectively. In contrast, the LPC18:2 content was the highest in GIFs, accounting for 31.03% of total LPC. Among LPE, LPI, and LPS species, the content of LPE18:0, LPI18:1, and LPS16:0 were the most abundant in HM, comprising 37.57%, 21.05%, and 26.02% of their respective classes, respectively. However, LPS molecules were not detected in CIFs and GIFs, it might be due to their loss during processing. In both CIFs and GIFs, LPE18:2 and LPI18:0 were the predominant species.

3.3.4 Acyl chain unsaturation of phospholipids

Unsaturation analysis of major glycerophospholipids and SM was conducted (Figure 4). The results showed that PC molecules mainly had two degrees of unsaturation in HM. In contrast, the content of PC molecule with one degree of unsaturation was the highest in CIFs and GIFs, accounting for 29.51 % and 30.09 % of the total PC, respectively. For PE, seven degrees of unsaturation were identified in HM, showing a greater variety compared to CIFs and GIFs. This difference might be due to the higher content of PUFA-containing PE molecules, such as those with DHA and EPA. Among all samples, PE molecules with two degrees of unsaturation were the most common, accounting for 53.05 %, 52.43 %, and 54.97 % of total PE in HM, CIFs, and GIFs, respectively. Moreover, PI and PS molecules, both with one degree of unsaturation, were the predominant species in CIFs and GIFs, while the content of saturated PS molecules was the highest in HM. SM molecules exhibited fewer unsaturation degrees than glycerophospholipids. Across all samples, the content of SM molecules with one degree of unsaturation was the highest, accounting for 42.69 %, 60.79 %, 67.94 % of total SM in HM, CIFs, and GIFs, respectively. These significant differences in acyl chain unsaturation between HM and infant formulas likely affect the formation of the milk fat globule membrane, potentially affecting milk fat absorption.

3.4 Difference analysis

To further investigate the differences in phospholipid composition among samples, PCA and OPLS-DA were carried out on HM and infant formulas (Figure 3a). The unsupervised PCA model showed a clear separation between HM and infant formulas, with no overlap, indicating significant differences in phospholipid profiles. Additionally, infant formulas grouped into four distinct clusters, corresponding to CIFG1, CIFG2, GIFG1, and GIFG2, suggesting that SBP supplementation significantly affected the phospholipid composition of infant formulas. Subsequently, OPLS-DA, a supervised discriminative model, was used to identify differential phospholipid molecules among HM, CIFs, and GIFs (Figure S1 a,c,e,g,i). There were 69 different phospholipid molecules (VIP value >1 and $P<0.05$) between HM and CIFs (Figure S1b), and 71 phospholipid molecules (VIP value >1 and $P<0.05$) were identified between HM and GIFs (Figure S1d). PC38:2 and PC39:5 were the most significant differential phospholipid molecules in HM, CIFs, and GIFs, respectively. In comparisons between CIFs and GIFs, 39 differential molecules were identified, including SM34:1, SM39:1, and PC30:0 (Figure S1f). Furthermore, the impact of SBP addition on phospholipid composition was analyzed. The results showed that there were 27 differential molecules between CIFG1 as well as CIFG2 (Figure S1h) and 28 between GIFG1 and GIFG2, all with VIP values greater than 1 ($P<0.05$) (Figure S1j).

3.5 The similarity score of infant formulas

Infant formulas serve as an essential substitute for breast milk, with their composition closely linked to infant growth and development. Hence, accurate evaluation of infant formulas is of great importance. Currently, evaluation models for HM lipids primarily focus on triglycerides, total fatty acids, and Sn-2 fatty acids, whereas evaluation models specifically for HMPL are rare. In our

previous research, we established a three-dimensional evaluation model for HMPLs based on the Bray-Curtis similarity index, incorporating PLFAs, phospholipid classes, and phospholipid molecules. However, HMPL composition is influenced by various factors, including lactation stage, maternal diet, and health status, resulting in fluctuations of HMPL levels. Thus, a more comprehensive and dynamic evaluation approach is necessary. In this study, we observed that the phospholipid concentration in infant formulas was lower than that in HM. To enhance the accuracy of HMPL assessment, a new evaluation parameter—phospholipid concentration—was introduced, providing deeper insights into HMPL composition (Table 2). The phospholipid concentration was designated as the first similarity evaluation group, comprising six similarity indexes. The primary contributors to score reductions were SM and total phospholipid concentration. The similarity scores for CIFs and GIFs ranged from 5.83 to 23.85 and 0.22 to 10.03, respectively, with CIFs (average score: 15.17) exhibiting higher similarity to HM than GIFs (average score: 4.5). Notably, infant formulas containing SBP (CIFG1 and GIFG1) had lower deduction scores than those without SBP (CIFG2 and GIFG2), suggesting that SBP addition improved phospholipid similarity to HM. Regarding the relative content of phospholipid classes, SBP addition increased PC content in CIFG1 and GIFG1, resulting in lower similarity scores for other phospholipid classes, particularly PE and SM. The average similarity score for phospholipid classes was 11.19, 20.18, 11.02, and 19.17 for CIFG1, CIFG2, GIFG1, and GIFG2, respectively.

A total of 12 similarity indexes was composed of the PLFA evaluation group, with average similarity scores of 21.81, 20.48, 20.80, 23.08, 19.52, and 21.45 for CIFs, GIFs, CIFG1, CIFG2, GIFG1, and GIFG2, respectively. These values were clearly higher than the score for phospholipid concentration

and phospholipid classes, and phospholipid molecules. The main cause of score reduction in PLFAs was the decreased UFA content, suggesting that infant formulas could benefit from additional phospholipids supplementation with MUFA- and PUFA-enriched. The final evaluation group consisted of phospholipid molecules, which had 16 different similarity indexes, showing similarity score variations from 6.93 - 16.71 of CIFs and 5.50 - 17.05 of GIFs. Score deductions were mainly due to the differences in PC36:2, PE36:2, SM40:1, and SM42:2. Additionally, SBP supplementation in CIFG1 and GIFG1 led to further score reductions due to increased PC36:4 and PE36:4 levels, likely resulting from the enrichment of these molecules in SBP (Zhu, Fauconnier, et al., 2024). Their presence altered the phospholipid composition, reducing the relative content of other phospholipid molecules. Finally, the average comprehensive similarity score of CIFs (65.68) was higher than that of GIFs (53.62), which might be due to the differences in raw materials and manufacturing processes. Compared with GIFs, the production process of CIFs is more mature based on the surging market demand. Additionally, CIFs may contain milk fat globule membranes, which is a component rich in milk phospholipids, resulting in a higher score for CIFs. Overall, while the SBP supplementation effectively increased the phospholipid concentration in infant formulas, it decreased molecular similarity due to composition differences between SBP and HMPLs. Therefore, to further improve the phospholipid profile of infant formulas, greater incorporation of animal-derived phospholipids, such as milk-derived, egg-derived, and marine-derived phospholipids, should be considered. This is due to HMPLs not only play a structural role but also participate in the absorption and utilization of milk fat during the digestion of breast milk. After adding animal phospholipids, the phospholipid composition of infant formulas is more similar to that of breast milk compared the addition of plant-derived phospholipids, and further providing more high-content phospholipid molecules in HMPLs,

such as PC36:2, PE36:2, and SM40:1. This changed phospholipid profile will simulate and reconstruct milk fat globules, and further influence the lipid metabolism of infants.

4. Conclusion

Through the detection and evaluation of phospholipid composition in HM and various infant formulas, a total of 34 PLFA species were detected across all samples. The content of C16:0, C18:1n9c, C18:2n6c, and C18:0 were the highest in HM and infant formulas, accounting for more than 70 % of total PLFAs in HM, CIFs, and GIFs, respectively. The average total phospholipid concentration in HM was 19.53 mg/100mL, which was significantly higher than that in CIFs (10.88 mg/100mL) and GIFs (4.74 mg/100mL). Regarding phospholipid class composition, SM was the predominant phospholipid class in HMPLS, accounting for 40.96 % of total PL, while PC was the most abundant in infant formulas, comprising 51.33% and 52.82% of total PLs in CIFs and GIFs, respectively. Furthermore, 137, 86, and 77 phospholipid molecules were detected in HM, CIFs, and GIFs, respectively. In HMPLs, PC36:2 and SM40:1 were the most abundant phospholipid molecules, accounting for 23.02 % of total PC and 25.85 % of total SM, respectively. However, in infant formulas, PC34:1 was the predominant PC molecule, contributing 18.99 % and 18.58 % of total PC in CIFs and GIFs, respectively. Meanwhile, SM38:0 and SM34:1 were the most abundant SM species in CIFs and GIFs, respectively. In addition, the similarity evaluation model was applied to assess the phospholipid similarity between HMPLs and infant formulas. The results showed that the average overall similarity score of CIFs (65.68) was higher than that of GIFs (53.62). Although SBP addition improved the similarity score in terms of phospholipid concentration for CIFG1 and GIFG1 compared to CIFG2 and GIFG2, it led to a reduction in the similarity score for phospholipid

molecules, likely due to structural differences between SBP and HMPLs. In conclusion, these findings highlighted the differences in phospholipid composition between HM and infant formulas, and provided a fundamental data for improving infant formula design. Future research should not only focus on optimizing phospholipid concentration in infant formulas but also prioritize enhancing the structural similarity of phospholipid molecules to better mimic the composition of HMPLs.

Declaration of Interests: Author Hong Zhang, Xuebing Xu, and Ruihua Guo were employed by Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions: XP and YW: conceptualization and validation. HQZ: methodology. XW, SZ, HZ, and RG: software and investigation. HZ: formal analysis. XP, XX, HZ: resources. HQZ: data curation, writing—original draft preparation, and visualization. HQZ, MLF, HZ, XX, RG, SZ, XW, YW, XP, and JLv: writing—review and editing. XP and YW: supervision. XP: project administration. JLv, YW and XP: funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Table 1 The phospholipid fatty acid of human milk and infant formulas (%)

Fatty acids	HM	CIFs	CIFG1	CIFG2	GIFs	GIFG1	GIFG2
C4:0	0.04±0.02	0.08±0.05	0.08±0.05	0.07±0.05	0.04±0.04	0.04±0.04	0.04±0.04
C6:0	0.16±0.06	0.27±0.08	0.28±0.09	0.25±0.06	0.20±0.08	0.19±0.06	0.21±0.09
C8:0	0.27±0.16	0.44±0.15	0.44±0.17	0.45±0.11	0.42±0.15	0.40±0.14	0.44±0.15
C10:0	1.38±0.21	0.88±0.20	0.89±0.20	0.86±0.18	1.05±0.29	0.95±0.20	1.15±0.33
C11:0	0.46±0.22	0.14±0.14	0.16±0.12	0.10±0.15	0.11±0.08	0.09±0.08	0.13±0.07
C12:0	3.32±0.88	1.71±1.06	1.57±1.21	1.92±0.70	1.43±0.94	1.40±0.98	1.46±0.90
C13;0	0.08±0.03	0.06±0.05	0.04±0.03	0.08±0.05	0.07±0.05	0.08±0.07	0.06±0.03
C14:0	4.39±0.85	3.18±1.09	3.12±1.28	3.26±0.67	3.59±1.29	3.45±1.48	3.74±1.05
C14:1	0.13±0.08	0.17±0.08	0.18±0.10	0.17±0.05	0.19±0.15	0.15±0.13	0.24±0.16
C15:0	0.11±0.05	0.24±0.19	0.22±0.21	0.28±0.14	0.15±0.08	0.14±0.08	0.16±0.07
C15:1	1.05±0.21	6.53±5.54	7.23±5.24	5.43±5.80	8.84±2.71	8.81±2.56	8.88±2.86
C16:0	22.00±3.43	26.91±3.66	26.21±4.29	28.02±1.87	30.77±3.81	31.86±3.83	29.68±3.45
C16:1	1.01±0.31	0.42±0.17	0.41±0.19	0.44±0.13	1.20±1.06	1.14±1.15	1.27±0.97
C17:0	0.18±0.04	0.14±0.04	0.14±0.04	0.14±0.04	0.14±0.03	0.14±0.03	0.14±0.02
C17:1	0.10±0.03	0.04±0.04	0.04±0.04	0.03±0.02	0.06±0.06	0.07±0.08	0.06±0.03
C18:0	10.12±2.04	10.22±4.25	10.75±4.37	9.37±3.91	12.09±1.84	13.12±1.93	11.07±0.98
C18:1n9c	26.8±4.80	27.68±8.51	27.15±9.59	28.50±6.36	22.46±2.71	21.09±2.07	23.83±2.58
C18:2n6t	0.18±0.08	0.16±0.09	0.17±0.09	0.14±0.07	0.13±0.06	0.13±0.03	0.13±0.08
C18:2n6c	17.09±2.02	17.25±3.8	17.43±3.59	16.97±4.09	13.27±3.19	14.00±3.65	12.54±2.43
C18:3n6	0.08±0.03	0.05±0.01	0.05±0.02	0.05±0.01	0.03±0.01	0.03±0.01	0.03±0.01
C18:3n3	4.76±3.32	2.01±0.51	1.99±0.44	2.03±0.60	2.71±3.56	1.78±0.71	3.64±4.80
C20:0	0.07±0.02	0.13±0.05	0.12±0.03	0.13±0.06	0.10±0.03	0.11±0.03	0.10±0.02
C20:1n9	0.48±0.22	0.16±0.05	0.17±0.05	0.14±0.05	0.11±0.05	0.10±0.05	0.12±0.05
C20:2	0.24±0.07	/	/	/	0.01±0.01	0.01±0.01	0.01±0.01
C21:0	0.22±0.07	0.09±0.02	0.08±0.02	0.09±0.03	0.03±0.02	0.04±0.02	0.03±0.02
C20:4n6	0.65±0.14	0.55±0.15	0.52±0.14	0.60±0.15	0.40±0.08	0.35±0.07	0.44±0.07
C20:3n3	0.08±0.05	/	/	/	/	/	/
C22:0	0.07±0.03	0.05±0.04	0.06±0.04	0.03±0.03	0.02±0.02	0.02±0.02	0.02±0.01
C20:5n3	0.03±0.01	0.15±0.07	0.16±0.07	0.13±0.06	0.08±0.04	0.08±0.04	0.07±0.04
C22:1	0.20±0.22	/	/	/	0.03±0.04	0.01±0.02	0.04±0.05
C22:2	0.04±0.02	/	/	/	/	/	/
C24:0	/	0.02±0.03	0.02±0.03	0.02±0.03	/	/	/
C22:6n3	0.38±0.20	0.29±0.10	0.29±0.11	0.30±0.08	0.26±0.06	0.24±0.06	0.28±0.05
C24:1	0.03±0.02	/	/	/	/	/	/
SC-SFA[b]	0.20±0.07	0.34±0.12	0.36±0.13	0.32±0.09	0.24±0.11	0.23±0.10	0.25±0.12
MC-SFA	5.52±0.98	3.23±1.26	3.11±1.50	3.42±0.69	3.08±1.25	2.91±1.23	3.24±1.25
LC-SFA	37.17±5.37	40.97±6.99	40.74±7.79	41.34±5.49	46.91±4.69	48.87±4.48	44.94±4.03
MUFA	33.60±4.03	35.00±4.79	35.18±5.80	34.71±2.41	32.90±2.94	31.37±1.95	34.42±2.98
PUFA	23.53±3.34	20.46±4.44	20.62±4.16	20.21±4.84	16.88±5.39	16.61±4.38	17.14±6.23
UFA	57.12±4.88	55.46±7.21	55.8±8.23	54.93±5.17	49.78±4.20	47.99±3.78	51.57±3.81
n3	5.25±3.34	2.45±0.62	2.44±0.54	2.46±0.72	3.04±3.57	2.10±0.77	3.99±4.81

n6	18.00±2.03	18.01±3.89	18.17±3.69	17.75±4.17	13.83±3.23	14.51±3.72	13.15±2.47

a: HM, human milk; CIFs, cow milk-based infant formulas; GIFs, goat milk-based infant formulas; CIFG1, CIFs with soybean phospholipids; CIFG2, CIFs without soybean phospholipids; GIFG1, GIFs with soybean phospholipids; GIFG2, GIFs without soybean phospholipids, there are significant differences among different group ($P<0.05$);

b: SC-SFA(short-chain saturated fatty acid)=∑(C4:0, C6:0); MC-SFA (medium-chain saturated fatty acid)=∑(C8:0, C10:0, C11:0, C12:0, C13:0); LC-SFA(long-chain saturated fatty acid)= ∑(C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0); SFAs (saturated fatty acids) = ∑(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0); MUFA (monounsaturated fatty acid) = ∑(C14:1, C15:1, C16:1, C17:1, C18:1n9c, C18:1n9t, C20:1, C22:1, C24:1); PUFA(polyunsaturated fatty acids) = ∑(C18:2n6c, C18:2n6t, C18:3n6, C18:3n3, C20:2, C20:3n6, C20:4n6, C20:3n3, C20:4n6, C20:5n3, C22:2, C22:6n3); n3 = ∑(C18:3n3, C20:3n3, C20:5n3, C20:3n3, and C22:6n3); n6 = ∑(C18:2n6c, C18:2n6t, C18:3n6, C20:3n6, C20:4n6).

Table 2 The similarity score of different infant formulas

	CIFs	CIFG1	CIFG2	GIFs	GIFG1	GIFG2
PC	0.54±0.72	0.26±0.40	0.9±0.86	2.53±0.85	1.85±0.6	3.22±0.37
PE	0.98±0.76	0.75±0.69	1.27±0.74	2.83±0.65	2.4±0.65	3.26±0.20
PI	0.04±0.03	0.04±0.03	0.03±0.03	0.01±0.01	0.01±0.01	0.01±0.01
PS	0.00±0.01	0.01±0.01	0.00±0.00	0.00±0.01	0.00±0.01	0.00±0.01
SM	3.45±1.13	3.61±0.83	3.25±1.39	4.62±0.35	4.57±0.32	4.68±0.36
SUM	4.82±2.85	4.28±2.56	5.48±3.05	10.51± 1.68	9.39±1.56	11.63± 0.85
PC	4.46±1.76	5.86±0.61	2.72±0.99	5.24±2.40	7.18±1.53	3.3±1.28
PE	0.39±0.75	0.59±0.93	0.14±0.27	1.54±1.37	0.76±1.17	2.31±1.10
PI	0.08±0.09	0.11±0.09	0.03±0.06	0.10±0.12	0.14±0.09	0.06±0.13
PS	0.06±0.06	0.07±0.07	0.04±0.05	0.05±0.07	0.05±0.07	0.05±0.07
SM	4.83±3.2	7.18±2.24	1.89±1.14	2.98±3.09	5.84±1.63	0.11±0.25
C16:0	0.25±0.34	0.28±0.36	0.21±0.32	0.89±0.83	1.11±0.95	0.68±0.62
C18:0	0.22±0.31	0.26±0.36	0.17±0.22	0.08±0.18	0.17±0.23	0.00±0.00
C18:1n9c	0.49±0.84	0.75±1.02	0.16±0.31	0.04±0.11	0.07±0.15	0.00±0.00
C18:2n6c	0.19±0.47	0.11±0.32	0.29±0.6	0.56±0.80	0.56±0.79	0.57±0.81
C20:4n6	0.01±0.02	0.02±0.02	0.01±0.01	0.03±0.03	0.04±0.03	0.02±0.01
C20:5n3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C22:6n3	0.01±0.01	0.01±0.01	0.01±0.01	0.00±0.01	0.01±0.01	0.00±0.00
MUFA	0.28±0.92	0.51±1.19	0.00±0.00	0.01±0.04	0.03±0.06	0.00±0.00
PUFA	0.47±0.77	0.49±0.67	0.45±0.88	1.13±1.07	1.04±1.11	1.23±1.01
UFA	1.03±1.80	1.62±2.13	0.30±0.80	1.00±1.62	1.74±1.96	0.26±0.59
n3	0.03±0.04	0.03±0.03	0.03±0.04	0.09±0.14	0.05±0.04	0.14±0.18
n6	0.21±0.52	0.14±0.38	0.30±0.64	0.67±0.87	0.68±0.88	0.66±0.86
PC34:2	0.38±0.40	0.68±0.27	0.00±0.00	0.43±0.41	0.81±0.20	0.05±0.05
PC34:1	0.55±0.59	0.05±0.14	1.17±0.25	0.65±0.71	0.00±0.00	1.30±0.39
PC36:4	0.07±0.07	0.13±0.04	0.00±0.00	0.09±0.09	0.18±0.03	0.00±0.00
PC38:6	0.01±0.01	0.01±0.02	0.00±0.00	0.05±0.00	0.05±0.00	0.05±0.00
PC36:2	2.89±0.23	2.92±0.25	2.85±0.2	2.16±0.5	2.58±0.18	1.75±0.34
PC36:1	0.53±0.55	0.96±0.37	0.00±0.00	0.12±0.24	0.25±0.29	0.00±0.00
PC38:6	0.07±0.00	0.07±0.00	0.07±0.00	0.01±0.02	0.03±0.02	0.00±0.00
PE34:1	0.32±0.11	0.26±0.09	0.39±0.09	0.32±0.16	0.22±0.13	0.41±0.13
PE36:4	0.07±0.06	0.12±0.04	0.01±0.01	0.10±0.09	0.18±0.04	0.02±0.02
PE38:6	0.02±0.02	0.02±0.02	0.02±0.03	0.07±0.00	0.07±0.00	0.07±0.00
PE36:2	1.36±1.55	2.45±1.30	0.00±0.00	2.11±2.34	4.22±1.41	0.00±0.00
PE36:1	0.62±0.62	1.09±0.43	0.03±0.05	0.79±0.95	1.59±0.74	0.00±0.00
PE38:6	0.30±0.00	0.30±0.00	0.30±0.00	0.12±0.11	0.15±0.08	0.09±0.12
SM38:1	0.23±0.34	0.39±0.39	0.02±0.06	0.62±0.59	0.15±0.34	1.10±0.36
SM40:1	2.71±0.45	2.69±0.46	2.72±0.43	2.62±0.51	2.74±0.43	2.51±0.55
SM42:2	1.39±0.04	1.40±0.04	1.38±0.04	1.18±0.11	1.27±0.05	1.10±0.09
G1 phospholipid concentration	15.17± 5.00	16.05± 4.14	14.07± 5.72	4.50±3.38	6.79±3.07	2.21±1.71
G2 phospholipid classes	15.18± 5.12	11.19± 2.94	20.18± 1.81	15.09± 4.77	11.02± 2.82	19.17± 2.11
G3 phospholipid fatty acid	21.81± 4.42	20.8±4.76	23.08± 3.56	20.48± 4.74	19.52± 5.55	21.45± 3.52
G4 phospholipid molecules	13.51± 2.95	11.48± 2.50	16.05± 0.41	13.54± 3.60	10.52± 2.73	16.57± 0.49

G_{TOTAL}	65.68± 9.35	59.51± 6.87	73.38± 5.54	53.62± 9.55	47.85± 9.36	59.39± 5.31

a: CIFs, cow milk-based infant formulas; GIFs, goat milk-based infant formulas; CIFG1, CIFs with soybean phospholipids; CIFG2, CIFs without soybean phospholipids; GIFG1, GIFs with soybean phospholipids; GIFG2, GIFs without soybean phospholipids, there are significant differences among different group ($P<0.05$);

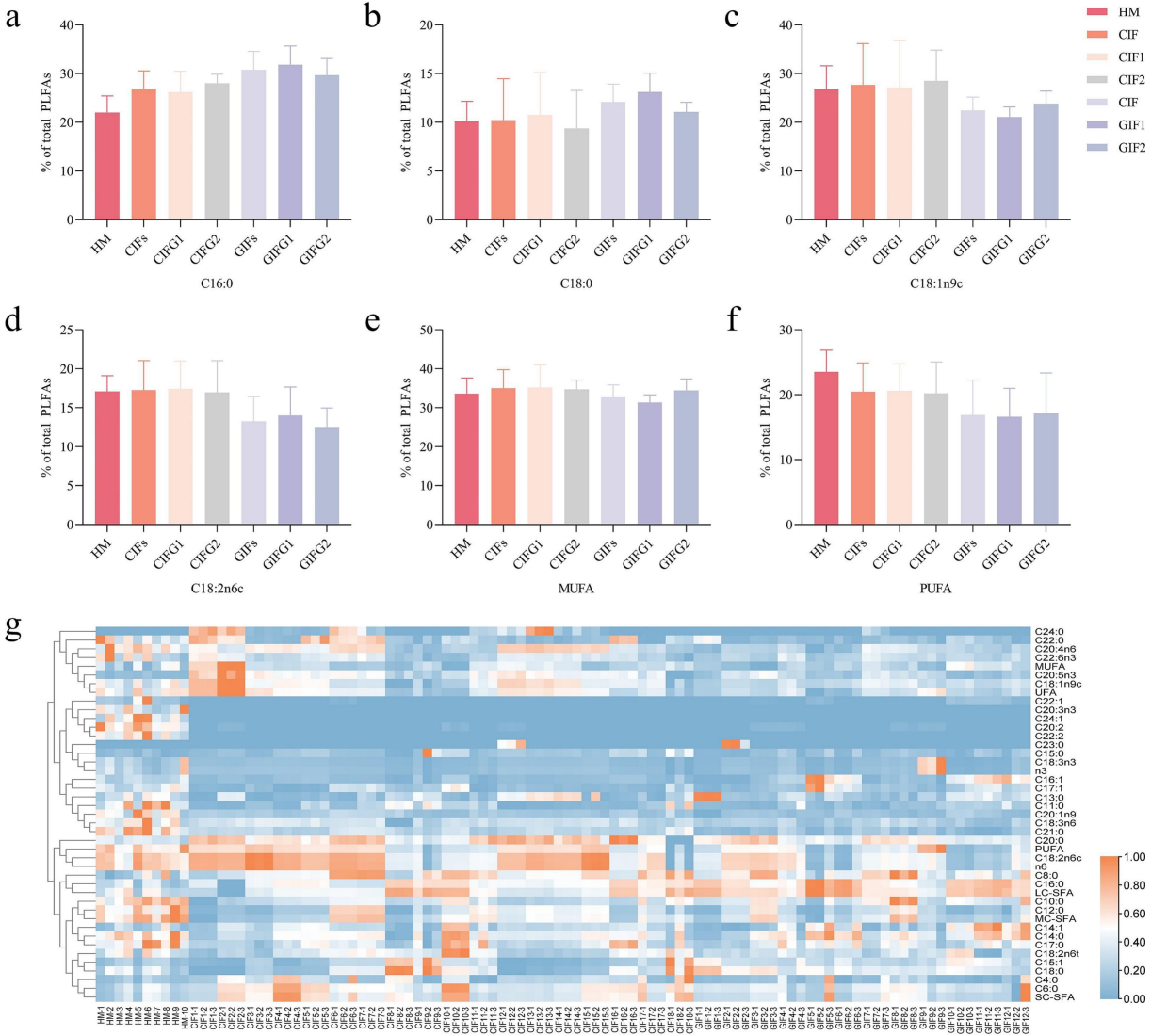
b: MUFA (monounsaturated fatty acid) = ∑(C14:1, C15:1, C16:1, C17:1, C18:1n9c, C18:1n9t, C20:1, C22:1, C24:1); PUFA(polyunsaturated fatty acids) = ∑(C18:2n6c, C18:2n6t, C18:3n6, C18:3n3, C20:2, C20:3n6, C20:4n6, C20:3n3, C20:4n6, C20:5n3, C22:2, C22:6n3); n3 = ∑(C18:3n3, C20:3n3, C20:5n3, C20:3n3, and C22:6n3); n6 = ∑(C18:2n6c, C18:2n6t, C18:3n6, C20:3n6, C20:4n6).

Declaration of interests

Author Hong Zhang, Xuebing Xu, and Ruihua Guo were employed by Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Highlight

1. There was significant difference between infant formula phospholipid with HMPL;
2. The dynamic and comprehensive evaluation model for phospholipid was established;
3. The similarity score of CIFs was higher than that of GIFs.


a
b
c
d
e
f
g
HM
CIF
CIF1
CIF2
CIF
GIF1
GIF2
% of total PLFAs
HM
CIFs
CIFG1
CIFG2
GIFs
GIFG1
GIFG2
C16:0
C18:0
C18:1n9c
C18:2n6c
MUFA
PUFA
C24:0
C22:0
C20:4n6
C22:6n3
MUFA
C20:5n3
C18:1n5c
UFA
C22:1
C20:3n3
C24:1
C20:2
C22:2
C23:0
C15:0
C18:3n3
n3
C16:1
C17:1
C13:0
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C8:0
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LC-SFA
C10:0
C12:0
MC-SFA
C14:1
C14:0
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C18:2n6t
C15:1
C18:0
C4:0
C6:0
SC-SFA
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0.60
0.40
0.20
0.00

Figure 1

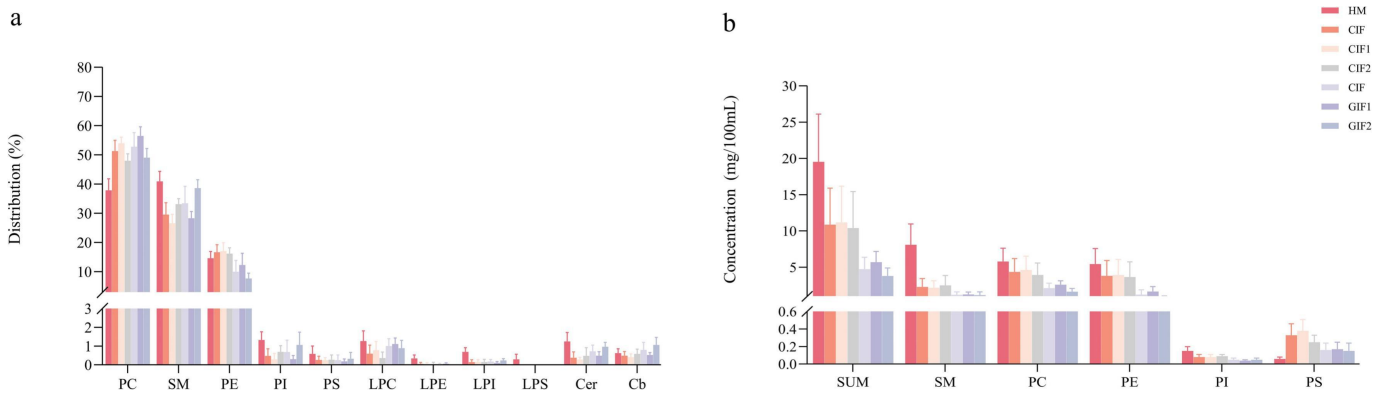
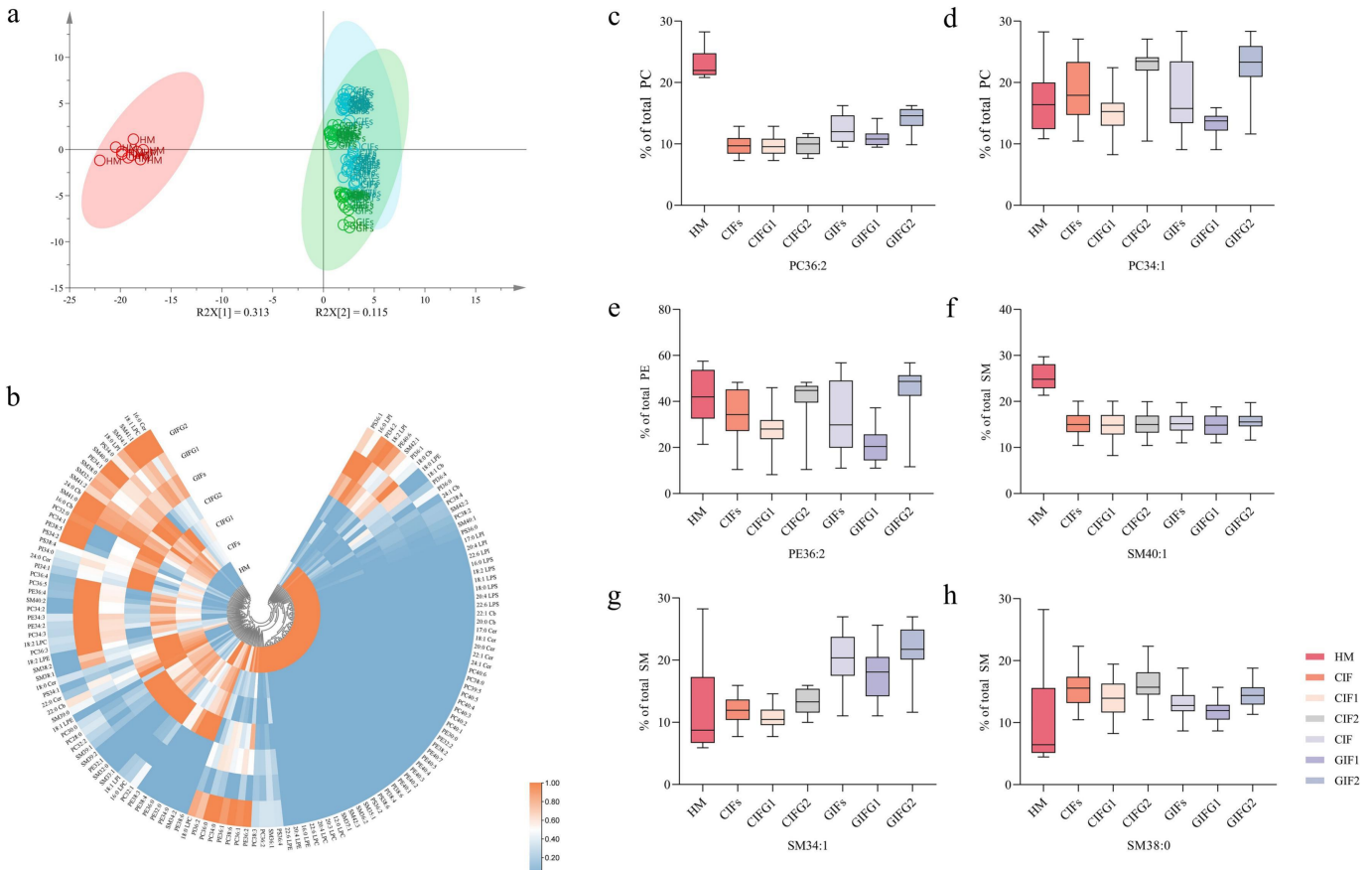

a
Distribution (%)
80
70
60
50
40
30
20
10
3
2
1
0
PC
SM
PE
PI
PS
LPC
LPE
LPI
LPS
Cer
Cb
b
Concentration (mg/100mL)
30
25
20
15
10
5
0.6
0.4
0.2
0.0
SUM
SM
PC
PE
PI
PS
HM
CIF
CIF1
CIF2
CIF
GIF1
GIF2

Figure 2


a
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R2X[2] = 0.115
HM
CIFs
CIFG1
CIFG2
GIFs
GIFG1
GIFG2
b
c
% of total PC
PC36:2
d
% of total PC
PC34:1
e
% of total PE
PE36:2
f
% of total SM
SM40:1
g
% of total SM
SM34:1
h
% of total SM
SM38:0
HM
CIF
CIF1
CIF2
CIF
GIF1
GIF2

Figure 3

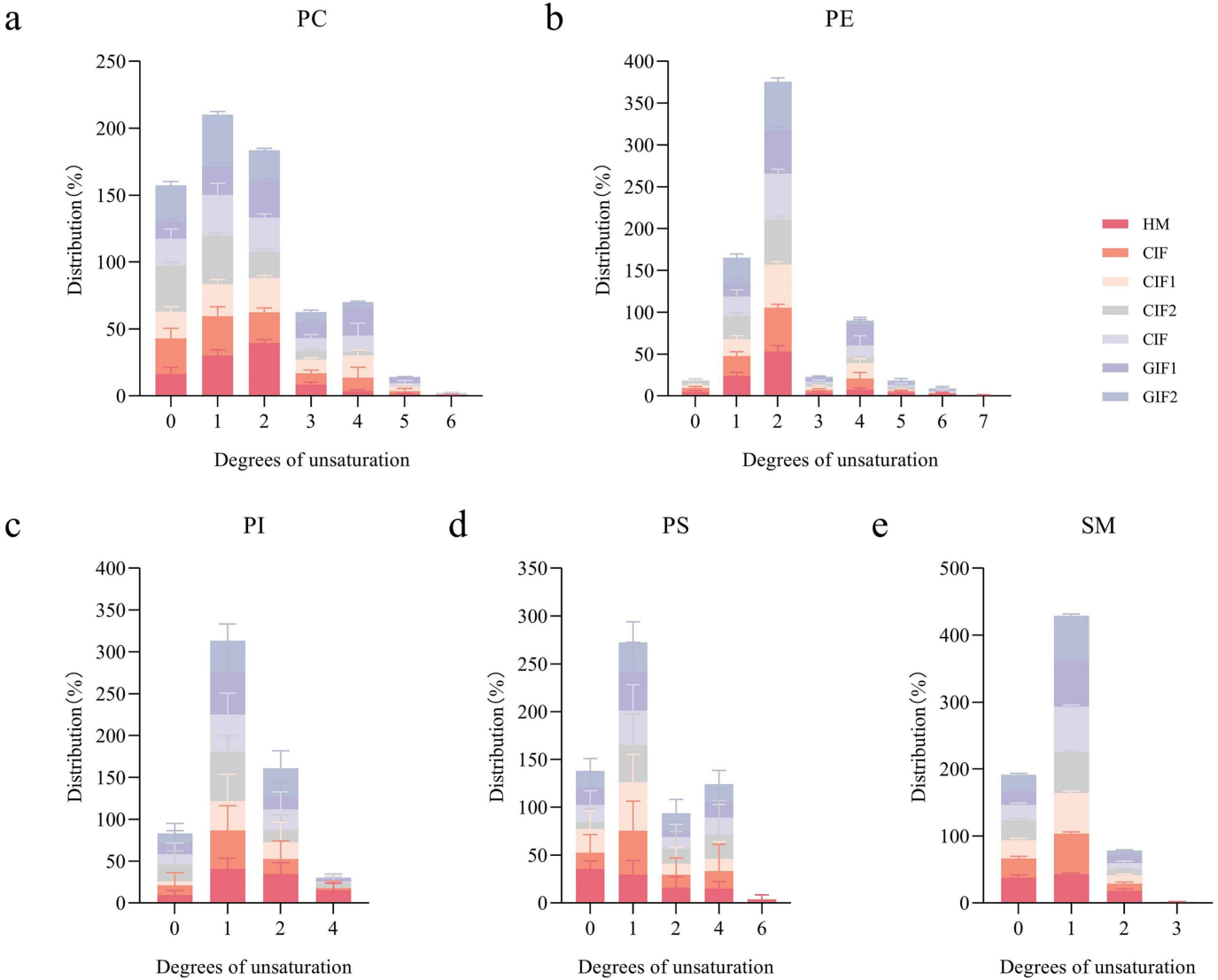
a
PC
Distribution (%)
Degrees of unsaturation
b
PE
Distribution (%)
Degrees of unsaturation
HM
CIF
CIF1
CIF2
CIF
GIF1
GIF2
c
PI
Distribution (%)
Degrees of unsaturation
d
PS
Distribution (%)
Degrees of unsaturation
e
SM
Distribution (%)
Degrees of unsaturation

Figure 4