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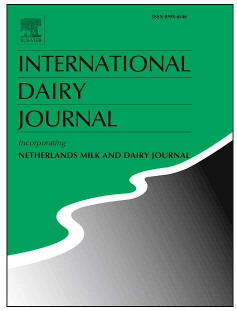

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Comparison of Comprehensive Fatty Acid Profile from different regions in Chinese Human Milk Project (CHMP) study

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Abstract: In this study, we aimed to detect the comprehensive fatty acid (FA) profile in human milk, including total fatty acids (total FAs), Sn-2 fatty acids (Sn-2 FAs), and phospholipid fatty acids (PLFAs), and further analyzed the influence of the sampling regions on human milk FA profile. The results showed that oleic acid (C18:1n9c), palmitic acid (C16:0), and linoleic acid (C18:2n6c) were the main FA species in total FAs, and Sn-2 C16:0 was the dominant Sn-2 FA species. Moreover, the PLFAs were mainly comprised of PL C16:0, PL stearic acid (PL C18:0), PL C18:1n9c, and PL C18:2n6c. Furthermore, FAs showed geographical differences, such as the highest content of linolenic acid (C18:3n3) and docosahexaenoic acid (C22:6n3) of total FAs was observed in Lanzhou and Weihai, respectively. Therefore, these results provide more FA data for the Chinese human milk database, and further enrich the theoretical information for the development of infant formula.

1. Introduction

Human milk is considered as the optimal food for the 0-6-month-old infants. Milk fat is the second largest (4 - 5 %) component of human milk, accounting for 50 % of necessary energy during the early stage of infant growth (Thum, et al., 2022). Human milk fat is mainly composed of triglycerides (TAGs, about 98 %), phospholipids (PLs, 0.8 - 2 %), cholesterol, sterol, and other bioactive lipids (Zhu, et al., 2023). Fatty acids (FA) are the basic components of TAGs and PLs, with more than 200 FA species being detected in human milk. The dominant FA species in human milk consisted of palmitic acid (C16:0), oleic acid (C18:1n9c), and linoleic acid (C18:2n6c), which accounted for 18.46 - 28.48 %, 28.14 - 34.71 %, and 17.30 - 25.42 % of total FAs, respectively (Bobiński & Bobińska, 2022; J. Jiang, et al., 2016; Peng, et al., 2009). Moreover, the FA content and species are affected by the lactation periods, the habitual diets, the physical conditions (age, parity, weights, etc.), and the geographic location of volunteer mothers. Most previous studies reported that the percentage of polyunsaturated fatty acids (PUFAs) in human milk increased with the extension of lactation periods (He, et al., 2021; W. Jiang, et al., 2020; Wang, et al., 2020), while a decreasing trend of PUFAs was demonstrated in another study (Zhao, et al., 2018). The monounsaturated fatty acids (MUFAs) showed the converse variation to PUFAs, whose content decreased from colostrum to mature milk (He, et al., 2021; W. Jiang, et al., 2020; Wang, et al., 2020).

It has been widely documented that more than 70 % of C16:0 is connected to the Sn-2 position of TAG in human milk, while the C16:0 is mainly located in the Sn-1 or Sn-3 positions in infant formula due to the wide use of vegetable oils, resulting in constipation or diarrhea caused by combining with calcium or magnesium when C16:0 is hydrolyzed at Sn-1,3 position (Ni, et al., 2022; Straarup, Lauritzen, Faerk, Hoy, & Michaelsen, 2006). Béghin (2019) reported that infant formula containing Sn-2 palmitate was safe, and it also could change fecal consistency (2 weeks to 2 months) and improve bone mineral content (at 4 months) (Béghin, et al., 2019). Another randomized clinical trial showed that infant formula abundant in Sn-2 palmitate enhanced the alpha diversity of gut microbes and acetate levels in infant feces, which was not significantly different from that in breastfed counterparts (Guo, et al., 2022). Moreover, for the impact factors of Sn-2 FAs, previous studies reported that the content of Sn-2 saturated fatty acids (SFAs) showed an increasing trend from colostrum to mature milk, while the contents of Sn-2 MUFAs and Sn-2 PUFAs decreased. Concerning the sampling region, the percentages of Sn-2 C16:0 and Sn-2 stearic acid (C18:0) in Wuhan were both higher than those in Zhengzhou and Harbin (Chen, et al., 2020).

Owing to biological activities, such as promoting infant brain development and accelerating the absorption and transfer of FAs, human milk PLs have been a focus of both researchers and infant formula manufacturers in recent years (Ali, et al., 2019; Liu, et al., 2022). PLs are primarily present in the milk fat global membrane consisting mainly of sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine (Zhu, et al., 2023). For the phospholipid fatty acids (PLFAs) in human milk, some researchers reported that the PL C16:0 is the most abundant PLFA species, accounting for 29.45 - 34.09 % of total PLFAs, followed by PL C18:0 (22.95 - 23.67 % of total PLFAs), PL C18:1 (13.03 - 13.79 % of total PLFAs), and PL C18:2 (11.79 - 12.04 % of total PLFAs) (Wei, et al., 2022; Yao, et al., 2016). Wei (2022) also found that the percentage of docosahexaenoic acid (C22:6n3, DHA) was 1.05 % of total PLFAs, which was higher than that in total FAs (Wei, et al., 2022). Furthermore, previous studies demonstrated that the PL PUFA percentage increased as the lactation period lengthened, while the content of PL MUFAs decreased (Zhang, et al., 2020; Zou, et al., 2012). Li (2022) found that the highest content for PL PUFAs was observed in Zhengzhou (Li, et al., 2020).

Although many studies have focused on human milk FAs, few studied investigated the specific influence of geographic region on the total FAs, Sn-2 FAs, and PLFAs, a factor that importantly influences the fatty acid profile. In this study, human milk samples were obtained from different sampling regions in China, including Zhengzhou (Central China), Lanzhou (Northwest China), Chengdu (Southwest China), Guangzhou (South China), Jinhua (East China), Beijing (North China), and Weihai (East China). The local people in these sampling regions have special daily diet habits, such as the local people in Guangzhou have abundant seafoods. The total FAs, Sn-2 FAs, and PLFAs in human milk were analysed. These results provide more comprehensive data on the FA profile of human milk, and further enrich the Chinese human milk database.

2. Materials and methods

2.1 Chemicals

Methanol (MeOH), chloroform ($CHCl_3$), and *n*-hexane were bought from Fisher Scientific (Pittsburgh, PA, USA). Anhydrous calcium chloride, pig pancreatin, pig bile salt, sodium chloride, calcium chloride, hydrochloric acid, and absolute ether were purchased from Sinopharm Chemical Reagent Co. LTD (Beijing, China), 37 fatty acid methyl ester (FAME) standards were provided by ANPEL Laboratory Technologies Inc. (Shanghai, China).

2.2 Human milk samples

The human milk samples were provided by the Chinese Breast Milk Project (CHMP), with the sample quantity and detailed sociodemographic information of volunteer mothers presented in Table 1. (This project has been registered on ClinicalTrials.gov with the identity number NCT03675204, and all procedures of this study were in accordance with the ethical standards formulated by Ethical committee of Shanghai Nutrition Association).

Human milk samples were obtained from different sampling regions, including Zhengzhou (n=33), Lanzhou (n=36), Chengdu (n=28), Guangzhou (n=25), Jinhua (n=30), Beijing (n=28), and

Weihai (n=20). Sampling was conducted between 60 to 180 d after birth. The breasts were thoroughly cleaned before the collection of milk, and sampling procedure was conducted between 9:00 and 11:00 a.m. Electric pumps were used to collect milk, with a minimum volume of 100 mL, and the milk samples were full breast milk. The milk samples were rapidly cooled with liquid nitrogen and stored at -80℃ for the further analysis. The voluntary mothers were provided with detailed information about the project and provided informed consent. The volunteer mothers were local residents who had no history of diabetes, infectious diseases, heart diseases, kidney diseases, and they also had no habits of smoking or alcohol consumption. The mean age of volunteer mothers was 34.94 ± 4.87 years old, with an average number of pregnancies of 1.39 ± 0.51. Furthermore, in order to eliminate the differences between individual samples (the effect of extreme values), the human milk samples of each city were mixed randomly into three mixing samples.

2.3 Extraction of human milk lipids

The milk lipids were extracted through the Folch method modified slightly (Folch, Lees, & Stanley, 1957). Briefly, 3 mL of human milk were mixed with 18 mL of extraction solution ($CHCl_3$/MeOH, 2/1, v/v). The resulting mixture was vortexed for 10 min, followed by mixing with 9 mL of 0.9 % (w/w) NaCl solution and shaking for 2 min. Following centrifugation at 1,166 × *g* for 15 min, the lower organic phase was collected and the organic solvent was removed using nitrogen blowdown evaporation (Allsheng Instrument Co., LTD, Hangzhou, China). The extracted lipids were stored at -80℃ for further analysis.

2.4 Preparation of Sn-2 monoglycerides

The milk lipids (30 mg) were dissolved in *n*-hexane (0.2 mL), and then pig pancreatin (30 mg, 100 - 400 U mg^{-1}), Tris-HCL buffer solution (2 mL, pH = 8), pig bile salt solution (0.5 mL, 1 g L^{-1}), and calcium chloride solution (2 mL, 220 g L^{-1}) were added for enzymolysis (37℃, 40 min). After enzymolysis, hydrochloric acid solution (1 mL, 6 mol L^{-1}) and anhydrous ether (1 mL) were added to the digested milk lipids and vortexed for 30 s, followed by centrifugation (1523 × *g*, 5 min). The upper phase containing the lipase product was collected and dried using nitrogen blowdown evaporation, followed by re-dissolving in 30 μL of *n*-hexane. The specific method of Sn-2 monoglyceride (Sn-2 MAG) separation was described in our previous study (Zhu, et al., 2022).

2.5 Extraction of phospholipids

The method for extracting PLs is based on a previous study, with modifications (Wei, et al., 2022). Briefly, breast milk PLs (50 mg) were dissolved in solution (1 mL, $CHCl_3$/MeOH, 2:1, v/v) and placed in an activated washing column (Silica gel bonded cartridges, CNWBOND Si, 1 g, 6 mL). First, 5 mL of solution 1 (*n*-hexane/diethyl ether, 50:1, v/v) and then 3 mL of solution 2 (*n*-hexane/diethyl ether, 6:1, v/v) were added to wash out nonpolar lipids. Next, 1 mL of solution 3 (*n*-hexane/diethyl ether, 1:1, v/v), 6 mL of solution 4 (MeOH), and 3 mL of solution 5 ($CHCl_3$/MeOH/Water, 3:5:2, v/v/v) were added to wash out polar lipids. The collected polar eluate was dried by nitrogen blowdown evaporation and stored at -80℃ for further analysis.

2.6 Fatty acid methylation

For the methylation of total FAs and Sn-2 FAs, breast milk lipids (20 mg) or Sn-2 MAG were dissolved by *n*-hexane (1 mL). Next, methanolic KOH solution (1 mol L^{-1}, 2 mL) was added, followed by vortexing for 10 min and centrifugation at 1523 × *g* for 10 min. The supernatant was collected and dried by the anhydrous sodium sulphate and then filtered through a 0.22 μm organic filter membrane for gas chromatography (GC) analysis. For methylation of milk PLs, BF_3-methanol (14 %, 300 μL) was pipetted into a tube containing the dried PLs, followed by heating at 100 ℃ for 90 min. After cooling to room temperature, heptane (600 μL) and saturated NaCl (500 μL) were added and the mixture was shaken. The supernatant was collected and filtered through a 0.22 μm organic filter membrane for GC analysis. The specific of GC conditions were reported in detailed in previous studies (X. Wang, et al., 2022; Zhu, et al., 2022).

2.7 Statistical analysis

Data analysis was carried out by SPSS 24.0 software (SPSS, Chicago, IL, USA) and expressed as mean ± standard deviation of three experiments (n = 3). Shapiro–Wilk and Levene's tests were performed to detect the normality and homogeneity of variance, respectively. In addition, the principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), accomplished by SIMCA-P software (Version 14.1, Demo Umetrics, Umea, Sweden), were used to resolve the ambiguous differences of total FAs, Sn-2 FAs, and PLFAs among different sampling cities. Finally, correlation analysis was applied to show the relationship among sociodemographic information of voluntary mothers, total FAs, Sn-2 FAs, and PLFAs.

3. Results and discussion

3.1 Total fatty acid profile

A total of 34 FA species were detected in the human milk from all samples, including 15 SFA species and 19 unsaturated fatty acid (UFA) species (Table 2). Among these FAs, the content of C18:1n9c was the highest, accounting for 32.88 - 34.63 % of total FAs, followed by C16:0 (18.65 - 25.11 % of total FAs), C18:2n6c (17.59 - 24.70 % of total FAs), C18:0 (5.8 - 8.13 % of total

FAs), C14:0 (3.77 - 5.23 % of total FAs), and C12:0 (3.15 - 4.51 % of total FAs). This observation was in accordance with previous results (Dao, Zhang, Wang, & Wang, 2023; He, et al., 2021; Wang, et al., 2020). Moreover, C18:1n9c, C16:0, and C18:2n6c showed the highest percentage in Chengdu, Guangzhou, and Zhengzhou, respectively ($P < 0.05$). For the remaining FA species, the percentage of DHA was 0.23 - 0.57 % of total FAs, and the highest DHA content (0.57 % of total FAs) was observed in Guangzhou. This result might be due to the voluntary mothers in Guangzhou having had higher daily intakes of sea food enriched with DHA. Geographic differences were also evident for linolenic acid (C18:3n3): the percentage of C18:3n3 of samples from Lanzhou (5.88 % of total FAs) was significantly higher than that in other cities (0.94 - 1.82 % of total FAs). Lanzhou was one of the major places of linseed production in China, correlating also with a high local consumption of linseed oil. Moreover, the contents of PUFAs, MUFAs, n3 FAs, and n6 FAs were 21.43 - 28.51 %, 32.90 - 37.39 %, 1.58 – 6.31 %, and 18.43 – 25.88 % of total FAs, respectively. The highest contents of PUFAs and n6 FAs were both observed in Zhengzhou, accounting for 28.51 % and 25.88 % of total FAs, respectively. Furthermore, the ratio of n6/n3 in Lanzhou was the lowest (3.36) among the sampled cities, owing to the content of n3 FA being the greatest in Lanzhou (6.31 % of total FAs).

To know the specific influence of the sampling regions on human milk FAs, we re-analysed previous results (last 5 years) (Supplementary Table S1). The C16:0 percentage in human milk ranged from 13.46 % to 22.53 % of total FAs in China, and its lowest content was reported in the northeast regions (Songyuan, Harbin, Changchun) (Wang, et al., 2020), while Chen et al. (2020) found the percentage of C16:0 in Harbin was the highest (22.53 % of total FAs). This different finding might be caused by the differences of milk samples and detection method. Moreover, compared with human milk obtained from mothers in The Philippines, the percentage of C16:0 in China was lower, and it was similar to that in Spain, Norway, and Finland. For other FA species, the proportions of C18:0 and C18:1n9c were 5.31 - 6.59 % and 27.74 - 34.53 % of total FAs, respectively, which was consistent with the results of the current research. However, the C18:0 content in Sichuan accounted for 9.61 % of total FAs and C18:1n9c content in northeast regions was 18.16 % of total FAs (Dao, et al., 2023; Wang, et al., 2020). The content of C18:2n6c in human milk from Chinese cities accounted for 16.82 - 46.06 % of total FAs, which was higher than that in human milk samples from Spain and The Philippines (9.30 - 15.38 % of total FAs) (Devaraj, et al., 2023; Sánchez-Hernández, et al., 2019). This significant difference might be the result of different dietary choices, with soybean and sunflower oils, both rich in linoleic acid (~50% and ~60%, respectively) being commonly used for cooking in China. Consequently, Chinese breast milk tended to have a higher linoleic acid content compared to that in other countries (Orsavova, Misurcova, Ambrozova, Vicha, & Mlcek, 2015; Wang, et al., 2020). The percentages of MUFAs and PUFAs, essential FAs for infant nutrition, were 20.58 - 40.53 % and 20.75 - 50.46 % of total FAs in Chinese cities, respectively. Moreover, breast milk in Spain had a higher MUFA content, accounting for 46.99 % of total FAs, while a lower PUFA percentage was observed in The Philippines (Devaraj, et al., 2023). This observation was primarily linked to the maternal diet in Spain, which was a part of the Mediterranean diet. The local population consumed a significant amount of olive oil, which contained of 63.30% oleic acid. Additionally, meat and cheese were also substantial sources of oleic acid (Barreiro, Díaz-Bao, Cepeda, Regal, & Fente, 2018; Chen, et al., 2020). The Southeast Asia was the main producing area for the palm oil, C16:0 content was the highest in palm oil, accounting for 44% of total FAs. For the Philippine's diet, with palm oil representing the main edible oil in The Philippines and frying being a favored way of cooking for local residents, the high intake of palmitic acid resulted in decreased intake of PUFAs, such as C18:2n6c.

3.2 Sn-2 fatty acid profile

Twenty-four Sn-2 FA species were detected in human milk, which were fewer than the species of total FAs (Table 3). Considering the distribution of Sn-2 FAs, a total of 10 Sn-2 FA species showed a higher percentage, which included Sn-2 caprylic acid (Sn-2 C8:0), Sn-2 capric acid (Sn-2 C10:0), Sn-2 C12:0, Sn-2 tridecylic acid (Sn-2 C13:0), Sn-2 C14:0, Sn-2 pentadecylenic acid (Sn-2 C15:1), Sn-2 C16:0, Sn-2 C18:0, Sn-2 C18:1n9c, and Sn-2 C18:2n6c. The Sn-2 C16:0 percentage was the highest in sampling cities, accounting for 44.12 - 53.68 % of total Sn2 FAs, which was in line with previous results (Chen, et al., 2020; He, et al., 2021). Among the remaining Sn-2 FA species, the content of Sn-2 C18:0 was the highest, taking up 7.37 - 10.15 % of total Sn-2 FAs, followed by Sn-2 C18:1n9c (4.57 - 9.27 % of total Sn-2 FAs), Sn-2 C14:0 (5.13 - 7.74 % of total Sn-2 FAs), Sn-2 C15:1 (5.90 - 8.04 % of total Sn-2 FAs), Sn-2 C13:0 (5.51 - 8.12 % of total Sn-2 FAs), and Sn-2 C18:2n6c (3.03 - 6.22 % of total Sn-2 FAs). Moreover, the Sn-2 FA content was also influenced by the sampling regions, with the highest Sn-2 C16:0 percentage being observed in Beijing (53.63 % of total Sn-2 FAs), while the lowest value was shown in Lanzhou, accounting for 44.12 % of total Sn-2 FAs. It was evident that the percentage of Sn-2 C12:0, Sn-2 C13:0, and Sn-2 C14:0 was the highest in Jinhua, and the maximum of Sn-2 C18:0 content was demonstrated in Weihai. The Sn-2 C18:1n9c and Sn-2 C18:2n6c both were observed the highest percentage in Lanzhou, which was similar to the results mentioned for total FAs in this research. For Sn-2 C22:6n3, it was only detected in Guangzhou and Weihai, accounting for 0.07 % and 0.12 % of total Sn-2 FAs, respectively. The reason for this observation might be caused by the lower content of Sn-2 C22:6n3 in other sampling regions, resulting that they failed to reach the minimum detection limit of GC.

Supplementary Table S2 shows the percentages of the main Sn-2 FA species determined in previous studies (Chen, et al., 2020; He, et al., 2021; López-López, López-Sabater, Campoy-Folgoso, Rivero-Urgell, & Castellote-Bargalló, 2002; Ni, et al., 2022; Qi, et al., 2018; Zou, et al., 2012). The percentage of Sn-2 C16:0 in human milk from Chinese cities varied from 48.21 % to 56.38 % of total Sn-2 FAs, concurring with the results of this current research (Chen, et al., 2020; He, et al., 2021; Ni, et al., 2022; Qi, et al., 2018; Zou, et al., 2012). It was reported that the C16:0 position at TAGs was extremely important for the growth and development of infants (Ni, et al., 2022). Some researchers found that the percentage of Sn-2 C16:0 was more than 50 % in human milk, and Sn-2 C16:0 monoglyceride was easily absorbed by the intestine of infants (Zhu, et al., 2021). Another research reported that infant formula with a higher level of Sn-2 palmitate improved the fine motor skills of infants, and its beneficial effects on infant neurodevelopment were associated with increased levels of intestinal *Bifidobacterium* (Wu, et al., 2021). It was reported that C18:0 preferentially connected to Sn-1 position of TAGs in comparison with C16:0 (Chen, et al., 2020). The Sn-2 C18:0 content was 1.31 - 4.32 % of total Sn-2 FAs in previous studies, whether in Chinese cities or foreign countries (Spain and Denmark), it was less compared with the results in this research. (López-López, et al., 2002; Zou, et al., 2012). Moreover, the Sn-2 C18:1n9c, Sn-2 C18:2n6c, and Sn-2 C18:3n3, Sn-2 PUFAs in previous literature showed higher contents. These differences were likely caused by the human milk samples obtained from different regions; another reason might be due to the difference among the time and temperature of enzymolysis in the detection process.

3.3 Phospholipid fatty acid profile

The PLs are important for the development of infants, accounting for 0.80 - 2.00 % of total human milk fat (Liu, et al., 2022; Zhu, et al., 2023). In this current research, a total of 30 PLFA species were detected (Table 4). The PL C14:0, PL C15:1, PL C16:0, PL C18:0, PL C18:1n9c, and PL C18:2n6c were the dominant PLFA species in the sampling regions. Moreover, similar to what we observed for Sn-2 FAs, the PL C16:0 content was the most abundant, accounting for 32.86 - 45.39 % of total PLFAs, which was consistent with the previous results (Wei, et al., 2022; Zhang, et al., 2020). The highest percentage of PL C16:0 was shown in Chengdu, while its lowest content was observed in Beijing. For other PLFA species, the PL C18:0 percentage was the dominant one, taking up 15.17 - 26.41 % of total PLFAs, followed by PL C18:1n9c (7.41 - 18.86 % of total PLFAs), PL C18:2n6c (8.07 - 14.78 % of total PLFAs), PL C14:0 (3.00 - 6.18 % of total PLFAs), and PL C15:1 (1.55 - 3.35 % of total PLFAs). The highest percentages of PL C18:1n9c and PL C18:2n6c were observed in Beijing, which accounted for 18.86 % and 14.78 % of total PLFAs. The PL C18:0 content in Zhengzhou was the highest (26.41 % of total PLFAs). In addition, PL C22:6n3 and PL C20:5n3 also showed regional differences: the proportion of both PL C22:6 and PL C20:5n3 was the highest in Beijing, accounting for 0.55 % and 0.11 % of total PLFAs, respectively. Compared with total FAs, PL C4:0 and PL C6:0 were not detected, this outcome might be due to the lower content of PL C4:0 and PL C6:0, resulting that they failed to reach the minimum detection limit of GC; another reason might be due to loss during the separating of PLs from human milk fat.

In previous studies, the PL C16:0 content was 20.20 - 37.36 % of total PLFAs in Chinese human milk samples (Supplementary Table S3), which was lower than that in this current research, while it was significantly higher than that in France and Finland (14.20 - 17.25 % of total PLFAs) (Benoit, et al., 2010; Fabritius, et al., 2020; Li, et al., 2020; Wei, et al., 2022; Zhang, et al., 2020; Zou, et al., 2013). The percentage ranges of PL C18:0, PL C18:1n9c, and PL C18:2n6c determined in previous researches were 14.84 - 29.42 % of total PLFAs, 10.86 - 23.60 % of total PLFAs, and 10.99 - 15.00 % of total PLFAs, respectively, in accordance with those found in this study. However, the proportions of PL C22:6n3 (0.37 - 1.05 % of total PLFAs), PL PUFA (15.90 - 22.20 % of total PLFAs), and PL MUFA (14.60 - 30.50 % of total PLFAs) in previous studies were all higher than those found in the current research (Table 3). These findings were likely due to the differences among the human milk samples, the PL detection method (mainly included GC, liquid mass spectrometry, and nuclear magnetic resonance method), and PL separating method (mainly included thin-layer chromatography and gradient elution). Thin-layer chromatography was one of the earliest method used for separating PL from total fat, and gradient elution utilizes the solubility of different lipids classes to achieve their separation, which was faster, greener and more efficient than thin-layer chromatography (Calvo, et al., 2020; Sánchez-Juanes, Alonso, Zancada, & Hueso, 2009). In addition, it was reported that after the dietary PLs were mainly digested in the intestine, and they were hydrolyzed into lysophospholipids and free FAs. Some lysophospholipids and free FAs were re-esterified into PLs and further bound to chylomicrons, and the rest was combined with low density lipoprotein (Lordan, Tsoupras, & Zabetakis, 2017).

3.4 Further analysis of total, Sn-2 and phospholipid fatty acids

PCA and PLS-DA were conducted to further analyze the influence of sampling regions on the total FAs, Sn-2 FAs, and PLFAs. After the PCA analysis, clear gaps were observed among different sampling regions for total FAs and PLFAs, while there were no obvious differences compared to Sn-2 FAs (Fig. 1 A, D, and G). This indicated that total FAs and PLFAs were more easily influenced by the sampling regions compared to Sn-2 FAs. For the total FAs, it was evident that the seven different cities were divided into two groups, one containing only Lanzhou and the other containing the remaining cities, indicating that the total FAs in Lanzhou was different from those in other cities. Lanzhou, located in the northwest of China, is characterized by a unique diet, rich in mutton, pasta and linseed oil. Considering the PLFAs, these cities were classified into three groups, the first group containing only Lanzhou, the second group comprising of Beijing and Weihai, and the third group including Guangzhou, Zhengzhou, Jinhua, and Chengdu. The PLS-DA loading diagrams were similar to the results of PCA analysis (Fig. 1 B, E, and H). Furthermore, the variable importance in projection (VIP) values of n6/n3, n6, C18:2n6c, PUFA, LC-SFA, C16:0, C18:3n3, n3, SFA, UFA, MUFA, C18:1n9c, C18:0, PL n6/n3, PL C18:0, PL C16:0, PL C15:1, PL C14:0, PL C18:1n9c, PL LCFA, PL MUFA, PL n6, PL C18:2n6c, PL PUFA, PL LC-SFA, PL C24:1, PL C20:1n9, PL SFA, PL UFA, PL C16:1, PL C24:0 were all more than 1 ($P<0.05$) (Fig. 1 C, F, and I), indicating that these FAs were potential biomarkers in distinguishing the different sampling regions and further enriched the Chinese human milk database.

The sociological information of volunteer mothers, including maternal age (year), parity, height, weight before pregnancy (kg), prenatal weight (kg), postnatal weight (kg), maternal body mass index (BMI) before pregnancy (kg m^{-2}), prenatal maternal BMI (kg m^{-2}), postnatal maternal BMI (kg m^{-2}), etc. also is an impact factor for human milk composition, However, the correlation analysis (Supplementary Fig. S1) revealed no strong correlation between the sociological information of volunteer mothers and total FAs, Sn-2 FAs and PLFAs of human milk. Interestingly, there were some strong correlations among different FA species. The C16:0 had strong positive relation with LC-SFA, C11:0, Sn-2 n6/n3, C17:0, and C22:6n3, and it had a strong negative correlation with Sn-2 MUFA, Sn-2 UFA, and Sn-2 C15:0. Furthermore, the PL C22:6n3 had a positive relation with PL C18:2n6t, PL C18:1n9c, and C15:0.

4. Conclusion

In conclusion, a total of 34, 24, and 30 FA molecular species were detected in the total FAs, Sn-2 FAs, and PLFAs in human milk, respectively. For total FAs, C18:1n9c was the most dominant FA species, accounting for 32.88 – 34.63 % of total FAs, while the percentage of Sn-2 C16:0 (44.12 – 53.68 % of total Sn-2 FAs) and PL C16:0 (32.86 – 45.39 % of total PLFAs) all were the highest in Sn-2 FAs and PLFAs, respectively. Moreover, the percentages of some fatty acid molecular species showed the regional difference, the highest content of C16:0 and PL C16:0 in both were shown in Chengdu, while the Sn-2 C16:0 content in Beijing was the maximum. The proportion of C18:3n3 was the highest in Lanzhou, whether in total FAs, Sn-2 FAs, or PLFAs, and the Sn-2 C22:6 was only detected in Guangzhou and Weihai, both of which were coastal cities. There was no obvious correlation among sociological information of volunteer mothers, total FAs, Sn-2 FAs, and PLFAs, while the positive correlation existed among the different FA species, such as the strong positive relation shown for the C16:0, LC-SFA, C11:0, and Sn-2 n6/n3. Therefore, these results not only provided more reference information for the Chinese breast milk database, but also provided theoretical data for the development of infant powder in the future.

Declaration of Interests: All 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.

Ethics Statement: The studies involving human participants were reviewed and approved by National Library of Medicine. The patients/participants provided their written informed consent to participate in this study.

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

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References：

Ali, A. H., Zou, X., Abed, S. M., Korma, S. A., Jin, Q., & Wang, X. (2019). Natural phospholipids: Occurrence, biosynthesis, separation, identification, and beneficial health aspects. *Critical Reviews in Food Science and Nutrition*, 59, 253-275.

Barreiro, R., Díaz-Bao, M., Cepeda, A., Regal, P., & Fente, C. A. (2018). Fatty acid composition of breast milk in Galicia (NW Spain): A cross-country comparison. Prostaglandins, *Leukotrienes and Essential Fatty Acids*, 135, 102-114.

Béghin, L., Marchandise, X., Lien, E., Bricout, M., Bernet, J.-P., Lienhardt, J.-F., Jeannerot, F., Menet, V., Requillart, J.-C., Marx, J., De Groot, N., Jaeger, J., Steenhout, P., & Turck, D. (2019). Growth, stool consistency and bone mineral content in healthy term infants fed sn-2-palmitate-enriched starter infant formula: A randomized, double-blind, multicentre clinical trial. *Clinical Nutrition*, 38, 1023-1030.

Benoit, B., Fauquant, C., Daira, P., Peretti, N., Guichardant, M., & Michalski, M.-C. (2010). Phospholipid species and minor sterols in French human milks. Food Chemistry, 120, 684-691.

Bobiński, R., & Bobińska, J. (2022). Fatty acids of human milk – a review. *International Journal for Vitamin and Nutrition Research*, 92, 280-291.

Calvo, M. V., Martín-Hernández, M. C., García-Serrano, A., Castro-Gómez, M. P., Alonso-Miravalles, L., García-Martín, R., Megino-Tello, J., Alonso, L., & Fontecha, J. (2020). Comprehensive characterization of neutral and polar lipids of buttermilk from different sources and its milk fat globule membrane isolates. *Journal of Food Composition and Analysis*, 86, 103386.

Chen, Y. J., Zhou, X. H., Han, B., Li, S. M., Xu, T., Yi, H. X., Liu, P., Zhang, L. W., Li, Y. Y., Jiang, S. L., Pan, J. C., Ma, C. H., & Wang, B. C. (2020). Composition analysis of fatty acids and stereo-distribution of triglycerides in human milk from three regions of China. *Food Research International*, 133, 109196.

Dao, X., Zhang, D., Wang, L., & Wang, L. (2023). Analysis of human milk fatty acid composition and its correlation with diet pattern (A study in Tibetan population gathering area). *Journal of Food Composition and Analysis*, 116, 105046.

Devaraj, S., Giuffrida, F., Hartweg, M., Estorninos, E. M., Buluran, K. B., Lawenko, R. B., Thakkar, S. K., & Samuel, T. M. (2023). Temporal evolution of fatty acid content in human milk of lactating mothers from the Philippines. *Prostaglandins Leukot Essent Fatty Acids*, 190, 102543.

Fabritius, M., Linderborg, K. M., Tarvainen, M., Kalpio, M., Zhang, Y., & Yang, B. (2020). Direct inlet negative ion chemical ionization tandem mass spectrometric analysis of triacylglycerol regioisomers in human milk and infant formulas. *Food Chemistry*, 328, 126991.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226, 497-509.

Guo, D., Li, F., Zhao, J., Zhang, H., Liu, B., Pan, J., Zhang, W., Chen, W., Xu, Y., Jiang, S., & Zhai, Q. (2022). Effect of an infant formula containing sn-2 palmitate on fecal microbiota and metabolome profiles of healthy term infants: A randomized, double-blind, parallel, controlled study. *Food and Function*, 13, 2003-2018.

He, G., Li, G., Jiang, Y., Hua, J., Chu, X., Xiong, L., Gong, J., Xiao, G., & Ye, X. (2021). Macronutrient content and fatty acid composition and their positional distribution in human breast milk from Zhejiang Province, China in different lactation periods. *Food Science & Nutrition*, 9, 6746-6761.

Jiang, J., Wu, K., Yu, Z., Ren, Y., Zhao, Y., Jiang, Y., Xu, X., Li, W., Jin, Y., Yuan, J., & Li, D. (2016). Changes in fatty acid composition of human milk over lactation stages and relationship with dietary intake in Chinese women. *Food & function*, 7, 3154-3162.

Jiang, W., Zhang, X., Cheng, J., Song, J., Jin, Q., Wei, W., Dong, M., & Wang, X. (2020). Variation of fat globule size and fatty acids in human milk in the first 30 days of lactation. *International Dairy Journal*, 100, 104567.

Li, S., Chen, Y., Han, B., Xu, T., Liu, T., Yi, H., Zhou, X., Zhang, L., Liu, P., Ma, C., Li, Y., Pan, J., & Jiang, S. (2020). Composition and variability of phospholipids in Chinese human milk samples.

International Dairy Journal, 110, 104782.

Liu, Q., Zhao, J., Liu, Y., Qiao, W., Jiang, T., Liu, Y., Yu, X., & Chen, L. (2022). Advances in analysis, metabolism and mimicking of human milk lipids. *Food Chemistry*, 393, 133332.

López-López, A., López-Sabater, M. C., Campoy-Folgoso, C., Rivero-Urgell, M., & Castellote-Bargalló, A. I. (2002). Fatty acid and sn-2 fatty acid composition in human milk from Granada (Spain) and in infant formulas. *European Journal of Clinical Nutrition*, 56, 1242-1254.

Lordan, R., Tsoupras, A., & Zabetakis, I. (2017). Phospholipids of Animal and Marine Origin: Structure, Function, and Anti-Inflammatory Properties. *Molecules*, 22, 1964.

Ni, M., Wang, Y., Yang, Z., Xu, X., Zhang, H., Yang, Y., Zhang, L., & Chen, J. (2022). Profiles of total and sn-2 fatty acid of human mature milk and their correlated factors: A cross-sectional study in China. *Frontiers in Nutrition*, 9.

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *International Journal of Molecular Sciences*, 16, 12871-12890.

Peng, Y., Zhou, T., Wang, Q., Liu, P., Zhang, T., Zetterström, R., & Strandvik, B. (2009). Fatty acid composition of diet, cord blood and breast milk in Chinese mothers with different dietary habits. Prostaglandins, *Leukotrienes and Essential Fatty Acids*, 81, 325-330.

Qi, C., Sun, J., Xia, Y., Yu, R., Wei, W., Xiang, J., Jin, Q., Xiao, H., & Wang, X. (2018). Fatty Acid Profile and the sn-2 Position Distribution in Triacylglycerols of Breast Milk during Different Lactation Stages. *Journal of Agricultural and Food Chemistry*, 66, 3118-3126.

Sánchez-Hernández, S., Esteban-Muñoz, A., Giménez-Martínez, R., Aguilar-Cordero, M. J., Miralles-Buraglia, B., & Olalla-Herrera, M. (2019). A Comparison of Changes in the Fatty Acid Profile of Human Milk of Spanish Lactating Women during the First Month of Lactation Using Gas Chromatography-Mass Spectrometry. A Comparison with Infant Formulas. *Nutrients*, 11, 3055.

Sánchez-Juanes, F., Alonso, J. M., Zancada, L., & Hueso, P. (2009). Distribution and fatty acid content of phospholipids from bovine milk and bovine milk fat globule membranes. *International Dairy Journal*, 19, 273-278.

Straarup, E. M., Lauritzen, L., Faerk, J., Hoy, C. E., & Michaelsen, K. F. (2006). The stereospecific triacylglycerol structures and fatty acid profiles of human milk and infant formulas. *Journal of Pediatric Gastroenterology and Nutrition*, 42, 293-299.

Thum, C., Wall, C., Day, L., Szeto, I. M. Y., Li, F., Yan, Y., & Barnett, M. P. G. (2022). Changes in Human Milk Fat Globule Composition Throughout Lactation: A Review. *Frontiers in Nutrition*, 9, 835856.

Wang, L., Li, X., Hussain, M., Liu, L., Zhang, Y., & Zhang, H. (2020). Effect of lactation stages and dietary intake on the fatty acid composition of human milk (A study in northeast China). *International Dairy Journal*, 101, 104580.

Wei, W., Li, D., Jiang, C., Zhang, X., Zhang, X., Jin, Q., Zhang, X., & Wang, X. (2022). Phospholipid composition and fat globule structure II: Comparison of mammalian milk from five different species. *Food Chemistry*, 388, 132939.

Wu, W., Zhao, A., Liu, B., Ye, W.-H., Su, H.-W., Li, J., & Zhang, Y.-M. (2021). Neurodevelopmental Outcomes and Gut Bifidobacteria in Term Infants Fed an Infant Formula Containing High sn-2 Palmitate: A Cluster Randomized Clinical Trial. *Nutrients*, 13, 693.

Yao, Y., Zhao, G., Xiang, J., Zou, X., Jin, Q., & Wang, X. (2016). Lipid composition and structural characteristics of bovine, caprine and human milk fat globules. *International Dairy Journal*, 56, 64-73.

Zhang, N., Zhuo, C.-F., Liu, B., Ye, W.-H., Tao, L., Zheng, L.-F., Chen, L., Deng, Z.-Y., Li, G.-Y., Gong, Z.-Q., & Li, J. (2020). Temporal Changes of Phospholipids Fatty Acids and Cholesterol in Breast Milk and Relationship with Diet. *European Journal of Lipid Science and Technology*, 122, 1900187.

Zhao, P., Zhang, S., Liu, L., Pang, X., Yang, Y., Lu, J., & Lv, J. (2018). Differences in the triacylglycerol and fatty acid compositions of human colostrum and mature milk. *Journal of Agricultural and Food chemistry*, 66, 4571-4579.

Zhu, H., Liang, A., Wang, X., Zhang, W., Zhang, Y., He, X., Liu, Y., Jiang, S., Lu, J., & Lv, J. (2021). Comparative Analysis of Triglycerides From Different Regions and Mature Lactation Periods in Chinese Human Milk Project (CHMP) Study. *Frontiers in Nutrition*, 8.

Zhu, H., Wang, X., Zhang, W., Zhang, Y., Zhang, S., Pang, X., Lu, J., & Lv, J. (2022). Dietary Schizochytrium Microalgae Affect the Fatty Acid Profile of Goat Milk: Quantification of Docosahexaenoic Acid (DHA) and Its Distribution at Sn-2 Position. *Foods*, 11, 2087.

Zhu, H., Wang, X., Zhang, W., Pan, J., Zhang, Y., Wang, Y., Jiang, C., Wei, Q., Si, X., Jiang, S., Lu, J., & Lv, J. (2023). Comparison of glycerophospholipid and sphingolipid in mature milk from different sampled regions in the Chinese human milk project (CHMP) study. *Food Chemistry*, 410, 135311.

Zou, X., Guo, Z., Huang, J.-H., Jin, Q.-Z., Cheong, L.-Z., Wang, X.-G., & Xu, X.-B. (2012). Human milk fat globules from different stages of lactation: a lipid composition analysis and microstructure characterization. *Journal of Agricultural and Food Chemistry*, 60, 7158-7167.

Zou, X., Huang, J., Jin, Q., Guo, Z., Liu, Y., Cheong, L., Xu, X., & Wang, X. (2013). Lipid composition analysis of milk fats from different mammalian species: potential for use as human milk fat substitutes. *Journal of Agricultural and Food Chemistry*, 61, 7070-7080.

Fig. 1 Score plots of PCA (A, D, G), PLS-DA (B, E, H), and VIP value plot of PLS-DA (C, F, I). The plot A, B, and C were produced by the total FAs, plot D, E, and F was conducted bySn-2 FAs, and plot G, H, and I was conducted by PLFAs. R2X and R2Y are the cumulative modeled variation in the X and Y matrix, respectively, and Q2 is the cumulative predicted variation in the Y matrix. PCA, principal component analysis; PLS-DA, partial least-squares-discriminate analysis; VIP, variable importance in projection; SC-SFA, short-chain saturated fatty acid; MC-SFA, medium-chain saturated fatty acid; LC-SFA (long-chain saturated fatty acid; SFAs, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; SCFA, short chain fatty acid; MCFA, medium chain fatty acid; LCFA, long chain fatty acid.

Supplementary Fig. S1 Correlation analysis among milk total fatty acid, Sn-2 fatty acids, phospholipid fatty acids, and sociodemographic information of voluntary mother in different sampled regions. The red unit indicated the positive correlation, in which the redder unit was the greater correlation. Similarity, the blue unit revealed the negative correlation, among which the green unit was the greater correlation; SC-SFA, short-chain saturated fatty acid; MC-SFA, medium-chain saturated fatty acid; LC-SFA, long-chain saturated fatty acid; SFAs, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; SCFA, short chain fatty acid; MCFA, medium chain fatty acid; LCFA, long chain fatty acid; BMI, body mass index.

Table 1 Sociodemographic information of volunteer mothers in seven cities

Sociodemographic information	Guangzhou n=25	Chengdu n=28	Weihai n=20	Beijing n=28	Jinhua n=30	Lanzhou n=36	Zhengzhou n=33
Maternal age (year)	35.88±3.76	34.03±4.37	34.95±3.01	38.18±3.14	35.1±4.09	33.78±3.02	35.09±3.49
Parity	1.46±0.5	1.21±0.41	1.4±0.49	1.43±0.49	1.47±0.56	1.14±0.35	1.61±0.55
Height (m)	1.6±0.05	1.6±0.04	1.64±0.04	1.63±0.05	1.6±0.05	1.63±0.05	1.62±0.04
Weight before pregnancy (kg)	51.96±6.81	50.28±6.37	57.98±7.46	55.75±7.05	57.43±15.98	53.81±5.2	55.18±7.35
Prenatal weight (kg)	64.29±7.45	62.83±6.93	73.78±8.58	70.82±8.68	71.75±19.31	67.43±6.58	70.3±8.88
Postnatal weight (kg)	60.44±6.44	59.42±7.84	55.45±6.39	59.41±7.12	57.48±6.3	58.55±7.16	60.3±6.38
Maternal BMI before pregnancy (kg m^{-2})	20.34±2.11	19.58±2.38	21.39±2.2	21.09±2.45	22.52±5.94	20.24±1.86	21.03±2.7
Prenatal maternal BMI (kg m^{-2})	25.16±2.14	24.49±2.79	27.25±2.83	26.77±2.76	28.19±7.51	25.38±2.51	26.78±3.09
Postnatal maternal BMI (kg m^{-2})	23.79±2.98	23.17±3.24	20.58±2.84	22.54±2.93	22.63±2.79	22.03±2.7	23.03±2.81
Collecting time (d)	60 - 180	60 - 180	60 - 180	60 - 180	60 - 180	60 - 180	60 - 180

BMI, body mass index

Table 2 The percentage of total fatty acid in different sampled regions (%)

Fatty acids	Beijing	Chengdu	Guangzhou	Jinhua	Lanzhou	Weihai	Zhengzhou
C4:0	0.04±0.00^{ab}	0.04±0.01^{ab}	0.05±0.01^{b}	0.02±0.01^{ac}	0.01±0.00^{c}	0.05±0.01^{b}	0.03±0.01^{ab}
C6:0	0.04±0.00^{a}	0.03±0.01^{a}	0.03±0.01^{ab}	0.01±0.01^{c}	0.01±0.00^{ac}	0.04±0.00^{b}	0.03±0.01^{b}
C8:0	0.09±0.00^{a}	0.09±0.01^{a}	0.07±0.02^{ab}	0.05±0.01^{b}	0.09±0.01^{a}	0.14±0.01^{c}	0.11±0.01^{ac}
C10:0	0.83±0.09^{ab}	0.77±0.06^{a}	0.86±0.13^{ab}	0.81±0.03^{a}	0.96±0.08^{ab}	0.81±0.07^{a}	1.01±0.05^{b}
C11:0	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{b}	0.01±0.00^{b}	0.02±0.00^{a}	0.00±0.00^{c}
C12:0	3.58±0.28^{ab}	3.15±0.36^{a}	3.83±0.56^{b}	3.78±0.26^{b}	4.51±0.19^{c}	3.3±0.35^{a}	4.22±0.02^{c}
C13;0	0.02±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00
C14:0	4.32±0.17^{a}	3.77±0.54^{b}	4.33±0.52^{a}	4.67±0.42^{ac}	5.23±0.35^{c}	4.00±0.31^{a}	4.54±0.17^{ac}
C14:1	0.08±0.01^{a}	0.03±0.00^{b}	0.05±0.01^{ab}	0.04±0.01^{b}	0.06±0.01^{ab}	0.07±0.00^{a}	0.05±0.00^{ab}
C15:0	0.18±0.01^{a}	0.13±0.01^{b}	0.15±0.02^{ab}	0.13±0.01^{b}	0.16±0.01^{ab}	0.17±0.02^{ab}	0.14±0.01^{b}
C15:1	0.19±0.03	0.21±0.01	0.23±0.02	0.20±0.07	0.21±0.03	0.27±0.05	0.22±0.03

C16:0	22.51±0.96[a]	24.65±1.17[b]	25.11±0.18[b]	23.1±1.16[ab]	18.65±0.48[c]	24.52±0.23[b]	21.64±0.44[ac]
C16:1	0.41±0.02	0.39±0.00	0.38±0.02	0.39±0.02	0.37±0.01	0.41±0.01	0.40±0.03
C17:0	0.28±0.03	0.25±0.01	0.27±0.05	0.23±0.01	0.25±0.00	0.26±0.01	0.24±0.00
C17:1	0.16±0.01[a]	0.12±0.01[b]	0.14±0.02[ab]	0.14±0.00[ab]	0.12±0.01[b]	0.12±0.01[b]	0.11±0.01[b]
C18:0	6.80±0.62[a]	8.13±0.31[b]	7.92±0.16[b]	6.75±0.54[a]	5.80±0.28[c]	7.20±0.12[ab]	6.19±0.15[ac]
C18:1n9c	33.21±1.01[ab]	34.63±0.98[a]	33.91±0.28[ab]	32.88±1.07[ab]	34.37±0.44[a]	33.09±0.48[ab]	31.45±0.95[b]
C18:2n6t	0.03±0.00	0.04±0.00	0.04±0.00	0.03±0.00	0.03±0.00	0.05±0.00	0.03±0.00
C18:2n6c	22.94±0.25[a]	17.59±0.58[b]	18.25±1.26[b]	21.77±2.01[a]	19.87±0.63[ab]	20.77±1.04[ab]	24.7±1.18[c]
C18:3n6	0.14±0.00[ab]	0.12±0.01[a]	0.11±0.01[a]	0.14±0.01[ab]	0.11±0.00[a]	0.16±0.01[b]	0.18±0.01[b]
C18:3n3	1.34±0.16[a]	1.70±0.30[b]	0.88±0.11[c]	1.82±0.18[b]	5.88±0.91[d]	0.94±0.12[c]	1.73±0.11[b]
C20:0	0.23±0.03[a]	0.23±0.02[a]	0.25±0.01[a]	0.17±0.01[b]	0.19±0.01[b]	0.33±0.03[c]	0.25±0.04[a]
C20:1n9	0.44±0.04[a]	1.08±0.34[b]	0.59±0.09[a]	0.59±0.03[a]	0.79±0.08[ab]	0.68±0.14[a]	0.46±0.08[a]
C20:2	0.46±0.02[a]	0.49±0.02[ab]	0.55±0.02[b]	0.50±0.01[ab]	0.37±0.00[c]	0.52±0.03[ab]	0.49±0.02[ab]
C20:3n6	0.33±0.01[a]	0.26±0.03[b]	0.33±0.03[a]	0.33±0.02[a]	0.25±0.01[b]	0.38±0.04[ac]	0.43±0.04[c]
C20:4n6	0.48±0.02[a]	0.43±0.01[ac]	0.50±0.01[ab]	0.50±0.02[ab]	0.40±0.01[c]	0.55±0.04[b]	0.54±0.02[b]
C20:3n3	0.05±0.01[a]	0.08±0.00[a]	0.06±0.01[a]	0.07±0.00[a]	0.13±0.02[b]	0.05±0.00[a]	0.05±0.01[a]
C22:0	0.04±0.01[a]	0.04±0.01[a]	0.05±0.01[a]	0.04±0.01[a]	0.07±0.01[b]	0.04±0.02[a]	0.04±0.00[a]
C20:5n3	0.12±0.02[a]	0.07±0.01[b]	0.12±0.01[a]	0.06±0.00[b]	0.07±0.00[b]	0.20±0.02[c]	0.12±0.03[a]
C22:1	0.10±0.04[a]	0.77±0.49[b]	0.13±0.05[a]	0.22±0.02[a]	0.48±0.06[c]	0.27±0.24[a]	0.16±0.06[a]
C22:2	0.01±0.00[a]	0.01±0.00[a]	0.03±0.01[b]	0.02±0.01[ab]	0.01±0.00[a]	0.02±0.00[ab]	0.01±0.00[a]
C24:0	0.13±0.02[a]	0.13±0.00[a]	0.15±0.03[ab]	0.15±0.01[ab]	0.17±0.01[b]	0.13±0.04[a]	0.12±0.00[a]
C22:6n3	0.38±0.07[a]	0.41±0.05[a]	0.57±0.14[b]	0.30±0.04[c]	0.24±0.02[c]	0.40±0.09[a]	0.23±0.02[c]
C24:1	0.04±0.02[a]	0.15±0.04[b]	0.04±0.01[a]	0.07±0.01[ab]	0.11±0.02[b]	0.05±0.01[a]	0.05±0.02[a]
SC-SFA	0.08±0.01[a]	0.07±0.01[a]	0.07±0.02[a]	0.04±0.02[b]	0.03±0.00[b]	0.09±0.01[a]	0.07±0.01[a]
MC-SFA	4.54±0.37[ab]	4.03±0.43[a]	4.79±0.71[b]	4.66±0.29[b]	5.59±0.25[c]	4.28±0.41[a]	5.37±0.07[c]
LC-SFA	34.49±1.41[a]	37.32±1.74[b]	38.24±0.56[b]	35.25±1.31[a]	30.52±0.54[c]	36.65±0.38[ab]	33.15±0.33[ac]
MUFA	34.63±1.09[a]	37.39±1.49[b]	35.47±0.39[a]	34.53±1.12[a]	36.51±0.33[ab]	34.96±0.69[a]	32.90±1.07[c]
PUFA	26.26±0.46[a]	21.18±0.70[b]	21.43±1.36[b]	25.53±2.13[a]	27.35±0.64[a]	24.03±1.24[ab]	28.51±1.19[a]
SFA	39.11±1.06[a]	41.43±2.15[a]	43.1±1.06[b]	39.94±1.02[a]	36.14±0.34[c]	41.01±0.63[a]	38.58±0.26[ac]
UFA	60.89±1.06[a]	58.57±2.15[ab]	56.9±1.06[b]	60.06±1.02[a]	63.86±0.34[c]	58.99±0.63[ab]	61.42±0.26[a]

SCFA	0.08±0.01^{a}	0.07±0.01^{a}	0.07±0.02^{a}	0.04±0.02^{b}	0.03±0.00^{b}	0.09±0.01^{a}	0.07±0.01^{a}
MCFA	4.54±0.37^{ab}	4.03±0.43^{a}	4.79±0.71^{b}	4.66±0.29^{b}	5.59±0.25^{c}	4.28±0.41^{ab}	5.37±0.07^{c}
LCFA	95.38±0.36	95.89±0.43	95.14±0.72	95.3±0.29	94.38±0.25	95.63±0.41	94.57±0.08
n3	1.87±0.19^{a}	2.26±0.27^{a}	1.63±0.10^{a}	2.24±0.19^{a}	6.31±0.92^{b}	1.58±0.16^{a}	2.14±0.10^{a}
n6	23.91±0.26^{a}	18.43±0.55^{b}	19.23±1.27^{b}	22.76±1.99^{a}	20.66±0.61^{ab}	21.91±1.08^{ab}	25.88±1.12^{c}
n6/n3	12.87±1.13^{a}	8.26±0.84^{b}	11.82±0.08^{a}	10.17±0.55^{ab}	3.36±0.62^{c}	13.99±1.07^{d}	12.10±0.28^{a}

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

Table 3 The percentage of Sn-2 fatty acid in different sampled regions (%)

Fatty acids	Beijing	Chengdu	Guangzhou	Jinhua	Lanzhou	Weihai	Zhengzhou
Sn-2 C4:0	0.41±0.13^{a}	0.67±0.06^{b}	0.73±0.07^{b}	0.59±0.30^{ab}	0.70±0.12^{b}	0.87±0.28^{c}	0.77±0.06^{b}
Sn-2 C6:0	0.31±0.07^{a}	0.58±0.07^{ab}	0.58±0.08^{ab}	0.32±0.17^{a}	0.62±0.11^{ab}	0.77±0.27^{b}	0.68±0.09^{ab}
Sn-2 C8:0	1.21±0.27	1.58±0.33	1.57±0.22	1.32±0.51	1.70±0.29	1.97±0.65	1.21±0.15
Sn-2 C10:0	1.04±0.08^{a}	1.67±0.07^{b}	1.05±0.04^{a}	1.33±0.36^{ab}	1.22±0.09^{ab}	1.70±0.21^{b}	1.29±0.11^{ab}
Sn-2 C11:0	0.27±0.03^{a}	0.67±0.11^{b}	0.51±0.14^{ab}	0.30±0.11^{a}	0.48±0.16^{ab}	0.58±0.09^{b}	0.47±0.11^{ab}
Sn-2 C12:0	2.09±0.20^{a}	1.66±0.10^{a}	3.19±0.37^{b}	3.10±0.67^{b}	2.79±0.24^{ab}	1.80±0.77^{a}	2.52±0.42^{ab}
Sn-2 C13:0	5.90±0.48^{a}	5.80±0.24^{a}	7.27±0.55^{b}	8.12±2.09^{b}	5.51±0.83^{a}	7.69±2.41^{b}	6.65±0.86^{ab}
Sn-2 C14:0	6.32±0.20^{a}	5.43±0.43^{b}	7.32±0.53^{c}	7.74±1.22^{c}	7.66±0.34^{c}	5.13±1.49^{b}	6.58±0.14^{a}
Sn-2 C15:0	0.27±0.03^{a}	0.27±0.07^{a}	0.20±0.06^{ab}	0.15±0.05^{b}	0.41±0.02^{c}	0.29±0.07^{a}	0.42±0.02^{c}
Sn-2 C15:1	5.90±0.53^{a}	6.55±0.59^{ab}	6.52±0.71^{ab}	6.80±2.52^{ab}	6.95±1.39^{ab}	8.04±2.96^{c}	7.26±1.16^{b}
Sn-2 C16:0	53.63±2.33^{a}	53.18±1.60^{a}	53.27±1.59^{a}	49.27±3.08^{ab}	44.12±1.55^{b}	47.67±4.90^{ab}	46.47±1.17^{ab}
Sn-2 C16:1	0.72±0.05^{a}	0.57±0.08^{b}	0.81±0.16^{a}	0.85±0.18^{a}	0.81±0.17^{a}	0.55±0.12^{b}	0.89±0.06^{a}
Sn-2 C17:0	0.25±0.05	0.21±0.01	0.22±0.05	0.20±0.02	0.24±0.01	0.19±0.05	0.21±0.02
Sn-2 C17:1	0.03±0.01^{a}	0.03±0.00^{a}	0.03±0.02^{a}	0.03±0.01^{a}	0.04±0.00^{ab}	0.02±0.01^{a}	0.05±0.01^{b}
Sn-2 C18:0	9.24±0.31	10.04±0.25	7.37±0.16	8.95±1.97	8.94±0.83	10.15±2.38	8.78±0.63

Sn-2 C18:1n9c	6.23±0.23^{a}	6.14±0.70^{a}	4.57±0.32^{b}	5.18±0.9^{b}	9.27±1.19^{c}	5.52±1.70^{ab}	7.87±0.92^{ac}
Sn-2 C18:2n9c	4.72±0.11^{a}	3.03±0.42^{b}	3.25±0.44^{b}	4.39±1.57^{a}	5.64±0.52^{c}	4.33±1.24^{a}	6.22±0.88^{d}
Sn-2 C18:3n3	0.13±0.03^{a}	0.12±0.06^{a}	0.06±0.02^{a}	0.13±0.07^{a}	1.25±0.38^{b}	0.24±0.19^{a}	0.25±0.05^{a}
Sn-2 C20:0	0.15±0.03^{ab}	0.14±0.01^{ab}	0.12±0.02^{ab}	0.09±0.03^{a}	0.08±0.00^{a}	0.18±0.03^{b}	0.14±0.04^{ab}
Sn-2 C20:1n9	0.23±0.04^{a}	0.42±0.11^{b}	0.21±0.01^{a}	0.17±0.02^{a}	0.46±0.05^{b}	0.45±0.18^{b}	0.37±0.03^{ab}
Sn-2 C20:4n6	0.48±0.11^{a}	0.52±0.04^{a}	0.51±0.06^{a}	0.47±0.09^{a}	0.29±0.02^{b}	0.94±0.08^{c}	0.32±0.13^{b}
Sn-2 C22:1	0.19±0.03^{a}	0.39±0.17^{b}	0.20±0.11^{a}	0.13±0.05^{a}	0.51±0.08^{c}	0.33±0.10^{ab}	0.29±0.06^{ab}
Sn-2 C22:2	0.28±0.03	0.32±0.02	0.39±0.06	0.35±0.12	0.32±0.07	0.47±0.26	0.29±0.03
Sn-2 C22:6n3	0.00±0.00^{a}	0.00±0.00^{a}	0.07±0.01^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.12±0.04^{c}	0.00±0.00^{a}
Sn-2 SC-SFA	0.72±0.19^{a}	1.25±0.13^{ab}	1.31±0.16^{ab}	0.91±0.47^{a}	1.32±0.24^{ab}	1.64±0.55^{b}	1.45±0.15^{ab}
Sn-2 MC-SFA	10.51±0.96	11.38±0.08	13.59±1.19	14.18±2.38	11.69±1.10	13.75±2.84	12.14±0.79
Sn-2 LC-SFA	69.86±1.92^{a}	69.28±1.77^{a}	68.5±1.41^{a}	66.4±3.31a^{b}	61.45±0.85^{b}	63.61±4.08^{b}	62.59±0.87^{b}
Sn-2 MUFA	13.30±0.84^{ab}	14.09±1.16^{ab}	12.34±0.34^{a}	13.16±1.54^{a}	18.05±0.45^{c}	14.91±1.61^{ab}	16.74±0.17^{b}
Sn-2 PUFA	5.61±0.25^{a}	4.00±0.49^{b}	4.26±0.36^{b}	5.35±1.55^{a}	7.50±0.73^{c}	6.10±1.18^{ac}	7.08±0.94^{ac}
Sn-2 SFA	81.09±0.87^{a}	81.91±1.59^{a}	83.4±0.18^{a}	81.49±1.40^{a}	74.46±0.83^{b}	78.99±1.05^{ab}	76.18±0.78^{ab}
Sn-2 UFA	18.91±0.87^{a}	18.09±1.59^{a}	16.60±0.18^{b}	18.51±1.40^{a}	25.54±0.83^{c}	21.01±1.05^{ac}	23.82±0.78^{ac}
Sn-2 SCFA	0.72±0.19^{a}	1.25±0.13^{ab}	1.31±0.16^{ab}	0.91±0.47^{ab}	1.32±0.24^{ab}	1.64±0.55^{b}	1.45±0.15^{ab}
Sn-2 MCFA	10.51±0.96^{a}	11.38±0.08^{ab}	13.59±1.19^{ab}	14.18±2.38^{ab}	11.69±1.10^{ab}	13.75±2.84^{ab}	12.14±0.79^{ab}
Sn-2 LCFA	88.77±1.15	87.37±0.20	85.10±1.34	84.91±2.85	87.00±1.33	84.61±3.39	86.41±0.94
Sn-2 n3	0.13±0.03^{a}	0.12±0.06^{a}	0.12±0.03^{a}	0.13±0.07^{a}	1.25±0.38^{b}	0.36±0.15^{a}	0.25±0.05^{a}
Sn-2 n6	0.48±0.11^{a}	0.52±0.04^{a}	0.51±0.06^{a}	0.47±0.09^{a}	0.29±0.02^{b}	0.94±0.08^{c}	0.32±0.13^{b}
Sn-2 n6/n3	3.70±0.49^{a}	5.15±2.10^{b}	4.47±1.61^{ab}	5.00±2.84^{b}	0.25±0.08^{c}	2.95±0.83^{ad}	1.29±0.39^{d}

Sn-2 SC-SFA(short-chain saturated fatty acid)=∑(C4:0, C6:0); Sn-2 MC-SFA (medium-chain saturated fatty acid)=∑(C8:0, C10:0, C11:0, C12:0, C13:0); Sn-2 LC-SFA(long-chain saturated fatty acid)= ∑(C14:0, C15:0, C16:0, C17:0, C18:0, C20:0); Sn-2 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,); Sn-2 MUFA (monounsaturated fatty acid) = ∑(C14:1, C15:1, C16:1, C17:1, C18:1n9c, C20:1, C22:1); Sn-2 PUFA(polyunsaturated fatty acids) = ∑(C18:2n6c, C18:3n3, C20:4n6, C22:2, C22:6n3); Sn-2 n-3 = ∑(C18:3n3, C22:6n3); Sn-2 n-6 = ∑(C18:2n6c, C20:4n6); Sn-2 SFA (saturated fatty acid) =∑(SC-SFA, MC-SFA, LC-SFA); UFA (unsaturated fatty acids)= ∑(MUFA, PUFA); Sn-2 SCFA (short chain fatty acid)= ∑(C4:0, C6:0); Sn-2 MCFA (medium chain fatty acid)= ∑(C8:0, C10:0, C11:0, C12:0, C13:0); Sn-2 LCFA (long chain fatty acid)= ∑(C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C14:1, C15:1, C16:1, C17:1, C18:1n9c, C20:1, C22:1, C18:2n6c, C18:3n3, C20:4n6, C22:2, C22:6n3)

Table 4 The percentage of phospholipid fatty acid in different sampled regions (%)

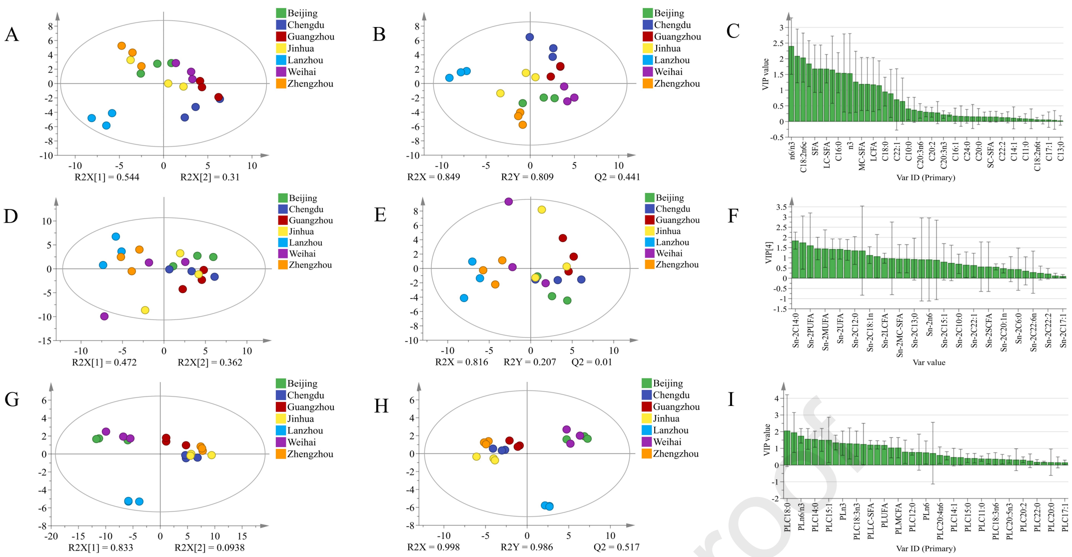
Fatty acids	Beijing	Chengdu	Guangzhou	Jinhua	Lanzhou	Weihai	Zhengzhou

PL C8:0	0.32±0.12[a]	0.11±0.02[b]	0.21±0.09[ab]	0.12±0.04[b]	0.13±0.02[b]	0.29±0.10[ab]	0.2±0.02[ab]
PL C10:0	0.59±0.09[ab]	0.51±0.01[a]	0.63±0.03[b]	0.58±0.02[ab]	0.64±0.00[b]	0.44±0.05[c]	0.73±0.02[d]
PL C11:0	0.17±0.06[ab]	0.16±0.02[ab]	0.24±0.01[a]	0.24±0.10[a]	0.13±0.01[b]	0.10±0.02[b]	0.32±0.05[c]
PL C12:0	1.70±0.04[a]	0.85±0.06[b]	1.22±0.09[ab]	0.96±0.13[ab]	2.29±0.09[c]	1.63±0.25[a]	0.98±0.00[ab]
PL C13:0	0.06±0.01[ab]	0.09±0.01[a]	0.10±0.01[a]	0.10±0.01[a]	0.07±0.00[ab]	0.04±0.00[b]	0.14±0.02[c]
PL C14:0	3.74±0.22[a]	3.00±0.13[b]	3.21±0.17[b]	3.73±0.38[a]	6.18±0.18[c]	3.96±0.22[a]	3.75±0.04[a]
PL C14:1	0.28±0.04[ab]	0.34±0.05[a]	0.38±0.02[a]	0.31±0.04[ab]	0.27±0.04[ab]	0.20±0.05[b]	0.60±0.02[c]
PL C15:0	0.11±0.01[b]	0.05±0.01[a]	0.04±0.01[a]	0.03±0.01[a]	0.07±0.01[ab]	0.15±0.02[c]	0.05±0.00[a]
PL C15:1	2.40±0.59[a]	1.55±0.19[a]	1.98±0.23[a]	1.84±0.28[a]	1.80±0.58[a]	2.08±0.27[a]	3.35±0.11[b]
PL C16:0	32.86±1.27[a]	45.39±0.33[b]	41.63±0.95[c]	44.31±0.76[bf]	35.61±0.49[d]	38.08±0.71[e]	43.47±0.15[f]
PL C16:1	1.09±0.12[a]	0.75±0.21[ab]	1.05±0.07[a]	0.77±0.18[ab]	0.95±0.27[a]	1.15±0.04[a]	0.52±0.02[b]
PL C17:0	0.26±0.01[a]	0.21±0.00[b]	0.21±0.00[b]	0.17±0.01[c]	0.22±0.00[b]	0.24±0.01[d]	0.19±0.01[c]
PL C17:1	0.07±0.01[a]	0.03±0.01[b]	0.04±0.01[b]	0.03±0.01[b]	0.05±0.00[b]	0.05±0.00[b]	0.04±0.00[b]
PL C18:0	16.83±1.31	23.32±0.86	22.78±1.08	25.4±1.16	18.53±0.77	15.17±1.34	26.41±0.15
PL C18:1n9c	18.86±1.57[a]	11.56±0.72[bc]	13.71±1.02[b]	9.67±1.27[c]	14.65±0.42[b]	17.83±1.13[a]	7.41±0.03[d]
PL C18:2n6t	0.58±0.04[a]	0.35±0.03[b]	0.42±0.03[ab]	0.32±0.05[b]	0.39±0.03[b]	0.65±0.03[a]	0.22±0.01[c]
PL C18:2n6c	14.78±1.37[a]	8.19±0.41[c]	8.69±0.64[c]	8.29±1.07[c]	12.58±0.27[b]	13.06±0.78[ab]	8.07±0.01[c]
PL C18:3n6	0.06±0.01[ab]	0.06±0.01[ab]	0.05±0.01[ab]	0.04±0.01[a]	0.06±0.00[ab]	0.08±0.01[b]	0.07±0.01[ab]
PL C18:3n3	0.73±0.07[a]	0.68±0.05[a]	0.34±0.02[b]	0.52±0.07[c]	2.81±0.08[d]	0.60±0.02[ac]	0.38±0.01[b]
PL C20:0	0.28±0.01[a]	0.26±0.03[ab]	0.25±0.04[ab]	0.20±0.03[b]	0.22±0.01[ab]	0.28±0.03[a]	0.25±0.01[ab]
PL C20:1n9	0.68±0.19[a]	0.67±0.09[a]	0.39±0.03[b]	0.32±0.05[b]	0.55±0.01[ab]	0.65±0.05[a]	0.37±0.01[b]
PL C20:2	0.28±0.04	0.20±0.01	0.26±0.01	0.18±0.03	0.19±0.02	0.29±0.02	0.18±0.01
PL C20:3n6	0.22±0.01[a]	0.16±0.01[b]	0.19±0.01[a]	0.15±0.02[b]	0.17±0.02[b]	0.23±0.01[a]	0.24±0.01[b]
PL C20:4n6	0.66±0.07[a]	0.50±0.03[ab]	0.51±0.04[ab]	0.45±0.06[b]	0.41±0.04[b]	0.56±0.05[ab]	0.64±0.01[a]
PL C22:0	0.03±0.01[a]	0.02±0.00[a]	0.03±0.01[a]	0.05±0.01[b]	0.04±0.00[b]	0.02±0.00[a]	0.05±0.01[b]
PL C20:5n3	0.11±0.02[a]	0.04±0.01[b]	0.07±0.01[c]	0.04±0.01[b]	0.03±0.00[b]	0.13±0.01[a]	0.06±0.01[c]
PL C24:0	0.30±0.24[a]	0.14±0.02[b]	0.14±0.01[b]	0.18±0.07[ab]	0.20±0.01[ab]	0.30±0.21[a]	0.11±0.01[b]
PL C22:6n3	0.55±0.05[a]	0.32±0.01[b]	0.39±0.04[bc]	0.25±0.02[d]	0.34±0.02[b]	0.43±0.03[c]	0.24±0.01[d]
PL C24:1	0.42±0.09[a]	0.06±0.01[b]	0.15±0.02[b]	0.18±0.12[b]	0.08±0.01[b]	0.20±0.08[b]	0.08±0.02[b]
PL MC-SFA	2.83±0.20[a]	1.72±0.01[b]	2.39±0.02[c]	2.02±0.05[d]	3.26±0.07[e]	2.51±0.19[ac]	2.36±0.07[c]

PL LC-SFA	54.49±2.53[a]	72.43±1.08[b]	68.32±1.92[bc]	74.11±1.62[b]	61.11±1.11[c]	58.27±2.03[ac]	74.31±0.29[b]
PL MUFA	23.80±1.15[a]	14.95±0.75[b]	17.70±1.22[c]	13.11±0.73[b]	18.36±0.71[c]	22.16±1.31[a]	12.38±0.20[b]
PL PUFA	17.98±1.62[a]	10.49±0.53[b]	10.92±0.77[b]	10.24±1.31[b]	16.99±0.42[a]	16.04±0.89[a]	10.09±0.05[b]
PL SFA	57.32±2.51[a]	74.15±1.08[bc]	70.72±1.91[b]	76.12±1.65[c]	64.37±1.03[d]	60.77±1.90[a]	76.68±0.22[c]
PL UFA	41.78±2.68[a]	25.44±1.27[bc]	28.62±1.99[c]	23.35±2.03[b]	35.34±1.13[d]	38.2±2.19[ad]	22.47±0.17[b]
PL MCFA	2.83±0.20[a]	1.72±0.01[b]	2.39±0.02[bc]	2.02±0.05[bc]	3.26±0.07[d]	2.51±0.19[c]	2.36±0.07[bc]
PL LCFA	96.27±0.46[a]	97.87±0.19[b]	96.95±0.07[ac]	97.46±0.43[bc]	96.45±0.04[a]	96.47±0.37a	96.78±0.11[ac]
PL n3	1.38±0.12[a]	1.04±0.07[b]	0.80±0.05[c]	0.81±0.09[c]	3.18±0.10[d]	1.17±0.04[b]	0.68±0.02[c]
PL n6	16.32±1.48[a]	9.24±0.46[b]	9.86±0.72[b]	9.25±1.19[b]	13.61±0.31[c]	14.58±0.86[ac]	9.23±0.03[b]
PL n6/n3	11.78±0.17[a]	8.88±0.28[b]	12.29±0.46[a]	11.39±0.33[a]	4.28±0.06[c]	12.45±0.68[a]	13.58±0.48[d]

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

Fig. 1


A
B
C
D
E
F
G
H
I
Beijing
Chengdu
Guangzhou
Jinhua
Lanzhou
Weihai
Zhengzhou
R2X[1] = 0.544
R2X[2] = 0.31
R2X = 0.849
R2Y = 0.809
Q2 = 0.441
R2X[1] = 0.472
R2X[2] = 0.362
R2X = 0.816
R2Y = 0.207
Q2 = 0.01
R2X[1] = 0.833
R2X[2] = 0.0938
R2X = 0.998
R2Y = 0.986
Q2 = 0.517
VIP value
Var ID (Primary)
VIP[4]
Var value

Declaration of interests

All 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.