TITLE PAGE

- Food Science of Animal Resources -

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Lipid Composition of Camel Milk and Cow Milk in Xinjiang Province of China Analyzed by Method of UPLC-Q-TOF-MS
Running Title (within 10 words)	Lipid of Camel Milk and Cow Milk in Xinjiang Province
Author	Jing Miao[1,2,3], Jun Wang[4]
Affiliation	1 Institute of Medicine of Xinjiang University, Xinjiang University, Urumqi, Xinjiang, PR China. 2 Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, Xinjiang University, Urumqi, Xinjiang, PR China 3 National Demonstration Center for Experimental Biology Education, Xinjiang University, Urumqi, Xinjiang, PR China 4 School of Life Science and Technology, Xinjiang University, Urumqi, Xinjiang, PR China
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Jing Miao (https://orcid.org/0000-0002-5441-6314) Jun Wang (https://orcid.org/0009-0007-7346-6580)
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research was supported by the Science and Technology Program from Xinjiang Uygur Autonomous Region (NO. 2020D01C029), the Scientific Research Program of University from Xinjiang Uygur Autonomous Region (XJEDU2018Y008), Tianchi Doctoral program in 2018 from the Xinjiang Uygur Autonomous Region, and the Science and Technology Program from Xinjiang University (BS180227). We also thank APTBIO SHANGHAI for its technical assistance during the determination of our milk samples in this manuscript.
Author contributions (This field may be published.)	Conceptualization: Jing Miao. Data curation: Jun Wang. Methodology: Jun Wang. Software: Jing Miao. Validation: Jun Wang. Investigation: Jing Miao. Writing - original draft: Jing Miao. Writing - review & editing: Jing Miao.
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.

Jing Miao **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Jing, Miao

ACCEPTED

Email address – this is where your proofs will be sent	miaojing3357122@126.com
Secondary Email address	Miaojing-1987@xju.edu.cn
Postal address	Xinjiang University, No. 777 Huarui Street, Shuimogou District, Urumqi, Xinjiang, China
Cell phone number	86-13579883936
Office phone number	None
Fax number	None

7

8

ACCEPTED

Authors and affiliation information should be listed on a separate Title Page.

Abstract

Xinjiang province is the main dairy production area of China, and Junggar Bactrian camel usually lived in the north part. Lipid is the main nutrient component of milk, and there is few reports about the differences in lipids between camel milk and cow milk in Xinjiang province. In this study, the analysis of lipids in Junggar Bactrian camel milk and cow milk in north part of Xinjiang province have been carried out by UPLC-Q-TOF-MS. As a result, 669 kinds of lipids are identified in total, which are divided into 16 lipid classes. In the results of multivariate statistical analysis, camel milk and cow milk can be separated definitely when analyzed by PCA, PLS-DA, and OPLS-DA, and revealed that lipids in camel milk is different from that in cow milk. Furthermore, 70 kinds of lipids are selected as differential lipids with the standards of fold change >2 or fold change <0.5, P <0.05, and VIP >1, which concludes 1 kinds of ceramides, 1 kinds of glycosphingolipids, 21 kinds of phosphatidylcholines, 10 kinds of phosphatidylethanolamines, 8 kinds of phosphatidylinositol, 8 kinds of phosphatidylserines, 11 kinds of sphingomyelins, and 10 kinds of triacylglycerides (TG). In the present study, the lipid profiles of camel milk and cow milk from Xinjiang province of China are disclosed, and it can provide foundation for the utilization of lipids from milk, as well as provide a potential reference for the camel milk and dairy products adulteration.

ACCEPTED

Keywords:

Camel milk; Cow milk; Lipidomics; Triacylglyceride

Introduction

Lipid is one of the most essential component in milk, which provide physical, sensory, and nutritional characteristics to dairy products, and consist of 3-5% (W/W) of milk (Bakry et al., 2021). Milk fat (MF) is the main component of milk lipid, and mainly comprises of triacylglycerides (TG), phospholipids, cholesterols, diacylglycerides (DG), monoglycerides (MGs), and free fatty acids. Due to the rich bioactive fatty acids, MF always play an important role in organisms, such as storing energy, forming cell membranes, and transmitting signals (Bang et al., 2017; Sioriki et al., 2016). MF also has anti-inflammatory properties against chronic diseases, such as obesity, cardiovascular diseases, cancer, and rheumatoid arthritis (Lordan & Zabetakis, 2017; Li, 2019).

In recent years, there are a number of studies on the differences between milk lipids of different species, which would be helpful for the further utilization and identification of dairy products. Now both cow milk and camel milk has been considered as potential functional foods for their plentiful fatty acids (Wang et al., 2022). As we all know, cow milk has become a daily food for human, and more and more people became to accept camel milk due to its good healthcare benefits as the production of camel milk increased year by year. Cow lipid mainly exists in the form of TG, DG, MGs, cholesterols, free fatty acids and phospholipids, which account for 97.5%, 0.36%, 0.02%, 0.31%, 0.02%, and 0.6% of total fat, respectively (Robert,

ACCEPTED

2002). However, the average lipid content in camel milk is 32.8±14.0 g/L, in which TG was the main lipid (96.24%), and the other lipids are cholesterol ester (0.1%), free cholesterol (0.84%), free fatty acid (0.65%), DG (0.7%), and phospholipid (1.2%) (Ali & Omar, 2001). In fact, camel milk produced at different lactation stages have been reported with different lipid compositions (Xiao, 2022). Furthermore, camel milk contains lower saturated fatty acids, higher unsaturated fatty acids (Maqsood et al., 2019), and higher polyunsaturated fatty acids (He et al., 2024) when compared with cow milk. Recent studies also shows that camel milk contains higher content of monounsaturated fatty acids than other kinds of milk (Ibrahim et al., 2023), and high levels of odd- and branched-chain fatty acids, as well as low ratios of n-6 to n-3 polyunsaturated fatty acids (Wang et al., 2022).

Xinjiang province is one of main dairy source area in China with vast area, and camel milk yield has reached 14,000 tons per year by 2019. In Xinjiang, all camels are raised in the desert and can freely consume plants that growing on deserts feeding. Now, more and more camel milk has been consumed with the rapid increase in the scale of camel pastured. In our former study, Junggar Bactrian camel milk and cow milk from different part of the north part of Xinjiang province have been found to have different fat contents, and cow milk showed lower fat and total solid contents than camel milk (Miao et al., 2023). Lipid is the most variable component of milk, and can be affected by many reasons, such as geography, breeds, lactation period, and season. However, people know few about the lipid profile of Junggar Bactrian camel milk in Xinjiang province. Therefore, the purpose of this study is to explain the lipid profiles of Junggar Bactrian camel milk and cow milk from the north part of Xinjiang province, and reveal differences between them, so as to better distinguish these two kinds of milk.

ACCEPTED

In this study, a non-targeted lipidomics analysis platform based on ultra-performance liquid chromatography quadrupole time of flight (UPLC-Q-TOF) system has been used for lipid identification and data processing of camel milk and cow milk, and subsequently some statistical analysis methods including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), orthogonal partial least squares discriminant analysis (OPLS-DA) and cluster analysis were used to select differential lipids between these two kinds of milk. These results would provide a comprehensive understanding of the lipid profiles of camel milk and cow milk from Xinjiang province of China. Our study shows the lipid profile of Junggar Bactrian camel milk and cow milk from the north part of Xinjiang province of China, as well as their differential lipids, which can provide foundation for the further utilization of lipids from camel milk, and provide a reference for the camel milk and dairy products adulteration.

Materials and Chemical reagents

Samples and reagents

All milk samples, contain 6 batches of Junggar Bactrian camel milk and 6 batches of Holstein cow milk, were collected from different areas of the north part of Xinjiang province, respectively, as listed in **Table 1**. Generally, camel always give birth every March and April, and entered mature lactation period from the 4th day to 320th day (Ming et al., 2023). During this period, camels lived in natural pasture, and freely consume plants in the pasture, such as camel thorn, and so on. All cow samples were collected from Holstein cows, which were fed with silage on farm.

Each batches of milk was collected as the mixture of milk from many camel or cow. These milk samples were collected in August of 2021, at which all Junggar

ACCEPTED

Bactrian camels were with mature lactation period. Milk samples were kept in clean milk storage bags laid in a 4℃ car-refrigerator on their return journey, and finally stored at -80℃ until analysis.

Acetonitrile (Thermo Fisher, Waltham, Massachusetts, USA) and methanol (Thermo Fisher, Waltham, Massachusetts, USA) of MS grade were used, while isopropanol (Thermo Fisher, Waltham, Massachusetts, USA), formic acid (Sigma, Santa Clara, California, USA), and ammonium formate (Sigma, Santa Clara, California, USA), methyl tert-butyl ether (Sigma, Santa Clara, California, USA) of chromatographic grade were all used.

Sample processing

All samples were processed according to the method of Xu et al. (2023). A milk sample of 30 mg was weighed precisely and transferred into a 2 mL centrifuge tube with appropriate magnetic beads, and 200 μL water pre-cooled at 4℃ in advance was added before they were flash freezed in liquid nitrogen for 5 s. And then a Fast Prep-24 homogenizer (MP, Santa Ana, California, USA) was used for 60 s at the rapid of 60 m/s, and this operation was repeated for three times. After that, 240 μL pre-cooled methanol was added and well-mixed in a Vortex mixer, and 800 μL methyl tert-butyl ether was added subsequently before they were well-mixed in a Vortex meter and further processed in an ultrasonic extractor at 4℃ for 20 min. And 30 min later, the mixture was centrifuged at 14000 rpm for 15 min at 10℃ in a low-temperature high-speed centrifuge. At last, the supernatant fluid was moved from the tube before dried with nitrogen and store at -80℃.

ACCEPTED

Each batches of camel milk and cow milk samples were extracted separately, and 3 batches of QC samples were prepared with equal amounts of all fourteen batches of milk samples at the same time for the evaluation of the analytical method.

Analytical methods

The UPLC Nexera LC-30A system (Shimadzu, Kyoto, Japan) together with an ACQUITY UPLC CSH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters, Milford, Massachusetts, USA) was employed for the separation of milk lipids. The column temperature was 45℃ with a flow rate of 300 μL/min and the injection volume of sample of 2 μL. The mobile phase consisted of A and B, while mobile phase A was 60% acetonitrile aqueous solution (V/V) containing 10 mM ammonium formate, and mobile phase B was 10% acetonitrile-isopropanol solution (V/V) containing 10 mM ammonium formate. The mobile phase was carried with the elution gradient as follows: 70% A and 30% B (0-2 min), 70-0% A and 30-100% B (2-25 min), while 70% A and 30% B (25-35 min). During the whole analysis, samples were stored in a 10℃ automatic injector and were injected according to a random sequence.

Mass data were recorded immediately by a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with both positive and negative ion modes of the Electrospray ionization (ESI). Heater temperature was set at 300℃, flow rate of sheath gas was 45 ARB, auxiliary gas was 15 ARB, sweep gas was 1 ARB, and capillary temperature was 350℃. For the positive mode, spraying voltage was 3.0 kV, S-lens RF level was 50%, and MS1 scan range was from 200 to 1800 m/z, while for the negative detection, spraying voltage was 2.5 kV, S-lens RF level was 60%, and Mass1 scan range was from 250 to 1800 m/z. Ten Mass2 scan were execute for each Mass1 scan, and survey scans were acquired at a resolution of 70000 at 200 m/z

ACCEPTED

for Mass1 scan, while the resolution of the HCD spectra was set to 17500 at 200 m/z for Mass2 scan.

Statistical analysis

Lipid Search TM software was used for the process of Mass data, which has been used as an automated lipidomics analysis software from Thermo Scientific, and recorded primary and secondary information databases of more than 1500000 kinds of lipids, including peak recognition, peak extraction, and searched against the software database for lipid identification. The precursor tolerance was 5 mg/kg, and product tolerance was 5 mg/kg, while product ion threshold was 5%. In order to accurately excavate the potential information in the date, univariate analysis and multivariate statistical analysis were applied using Metaboanalyst online software (https://www.metaboanalyst.ca/Metabo-Analyst/home.xhtml, updated on 1/18/2024). Furthermore, univariate statistical analysis was used to distinguish differential lipids between camel milk and cow milk, which mainly includes student's t-test/nonparametric test and fold change analysis, and multivariate statistical analysis includes PCA, PLS-DA and OPLS-DA.

Differential lipids between camel milk and cow milk were preliminary screened out by combining p-value and VIP value of OPLS-DA, and hierarchical cluster analysis of differential lipids was performed. The experiment of this study was mainly conducted by Applied Protein Technology Limited Company, SHANGHAI.

Results and Discussion

Evaluation of analytical method

ACCEPTED

The TIC spectrograms of three QC samples are compared, and the result shows that the chromatographic peak response intensity and retention time of each QC sample overlapped well both in positive and negative ion modes (**Figure S1**). Further analysis also shows that correlation coefficients of three batches of QC samples are all more than 0.999 (**Figure S2**), and three QC samples are closely clustered in PCA (**Figure S3**). Meanwhile, QC samples, camel milk samples, and cow milk samples all are analyzed by Hotelling T2 test, and confidence interval of three QC samples are within 99% (**Figure S4**). All these above results indicate that the analytical method used in this study is reliable, steady, defined, and repeatable.

Identification of lipids in cow and camel milk

Information of lipids identified in camel milk and cow milk are showed in **Figure 1A** and **Table S1**. Totally, 669 kinds of lipids are identified in both camel milk and cow milk, and these lipids can be described as 16 lipid classes, include 24 kinds of ceramides (Cer), 45 kinds of glycosphingolipids (CerG1), 1 kind of diglucose ceramide (CerG2), 31 kinds of DG, 12 kinds of lysophosphatidylcholines (LPC), 15 kinds of lysophosphatidyl- ethanolamine (LPE), 1 kinds of lysophosphatidylinositol (LPI), 69 kinds of phosphatidylcholines (PC), 61 kinds of phosphatidylethanolamines (PE), 6 kinds of phosphatidylglycerols (PG), 15 kinds of phosphatidylinositol (PI), 39 kinds of phosphatidylserines (PS), 50 kinds of sphingomyelins (SM), 5 kinds of sphingosines (So), 294 kinds of TG, and 1 kind of wax ester (WE).

Contents of 16 classes of lipids identified from camel milk and cow milk varied at different extent as listed in **Figure 1B**. All data are presented as mean±SD, and statistical and graphical evaluations are conducted by student's t-test. Contents of CerG1, CerG2, TG, and PG in cow milk are significantly higher than that in camel milk,

ACCEPTED

while numbers of other kinds of lipids in camel milk and cow milk do not show significant differences. When compared with other kinds of lipids, contents of TG identified from camel milk and cow milk is the highest. This result is same with other reports (Robert, 2002). In Alxa Bactrian camel milk, number of TG also is the highest, and followed by DG, PE and SM (Xiao, 2022), which is similar with our results. Moreover, content of TG in cow milk is higher than that in camel milk, and it means cow milk is more suitable for the production of infant formula milk powder than camel milk, because TG can well meet the energy requirements for the growth of infants and young children (Xiao, 2022).

Junggar Bactrian camel milk were analyzed in this study, and lipid in camel milk also can be affected by the different breeds of camel, as we all know. In Alxa Bactrian camel milk from different lactation periods, totally 980 kinds of lipids have been identified, and were divided into 24 classes (Xiao, 2022). Furthermore, 353 lipids were determined in milk fat globule membrane of Alxa Bactrian camel milk (He et al., 2024). However, although analytical method used in the present study is same with the literatures (Xiao, 2022; He et al., 2024), only 669 kinds of lipids have been detected in this study. Therefore, these great differences could be mainly ascribed to the differences of camel breed and living environment (Xiao, 2022).

Many kinds of fatty acid chains are included in lipids (**Table S1**), and these fatty acids contain 4 to 44 carbons, and the highest number of double bonds is up to 6. Among the lipids detected (**Figure 2A**), 299 kinds of fatty acids are identified, and type of occurrences of short-chain fatty acids is 63, while 21, 372 and 213 for types of medium-chain fatty acids, long-chain fatty acids, and very-long chain fatty acids, respectively. C16:0, C18:0 and C15:0 occur most frequently, and then followed by unsaturated fatty acids C16:1 and C18:1. Saturated fatty acids, especially C12:0, C14:0 and C16:0, are

ACCEPTED

associated with elevated cholesterol levels and increased risk of cardiovascular diseases (Sun et al., 2007). The unsaturation of unsaturated fatty acids is from 1 to 6 (**Figure 2B**). The polyunsaturated fatty acids have positive impacts on cardiovascular diseases, platelet aggregation, cancer, and various immune diseases (Siscovick et al., 2017).

According to former reports, camel milk contains lower saturated and higher unsaturated fatty acids, which help to the higher antioxidant activity and angiotensin-1 converting enzyme inhibitory potential after simulated gastro-intestinal digestion when compared to cow milk (Maqsood et al., 2019). Especially, content of unsaturated fatty acids in camel is 37.29%, and 14 kinds of fat acids have been determined from Alxa Bactrian camel milk, and the contents of oleic acid, stearic acid, and palmitic acid are 31.03%, 26.48% and 21.85%, respectively (Yun et al., 2013). Furthermore, palmitic acid also is considered as the feature fat acid of camel milk (Wen, 2023). In the present study, oleic acid, stearic acid, and palmitic acid have been detected in cow milk and camel milk, and they exist in the form of TG, DG, LPC, LPE, LPI, PC, PE, PI, PS, Cer, and SM, as showed. Most of them exist in the form of TG.

Multivariate statistical analysis of lipidomics in camel milk and cow milk

PCA is an unsupervised data analysis method, which can reflect the variability between and within groups. According to the result of PCA (**Figure 3A**), 6 batches of cow milk and 6 batches of camel milk are distinguished clearly. As listed in the OPLS-DA score plot (**Figure 3B**), the lipids of camel milk and cow milk are classified distinctly, and the parameter classifications are R2Y = 0.997, and Q2 = 0.958, which demonstrated that the model of used was credible and not overfitted. When analyzed by PLS-DA, these two different milk samples also are separated completely (**Figure 3C**), and the parameter classifications are R2Y = 0.998, and Q2 = 0.941 after a 5-fold

ACCEPTED

cross-validation, which indicated that the model used is proper. All these results tell that three statistical analysis method can distinguish camel milk from cow milk based on the lipids profiles, and lipids in camel milk are different from lipids in cow milk. In a former study, lipids in three kinds of milk samples have been distinguished using OPLS-DA model, and as a result human and cow milk can be distinguished correctly, while caprine and cow milk can not (Lina et al., 2020).

Identification of differential lipids between camel milk and cow milk

Differential lipids are selected by both univariate statistical analysis (Fold Change Analysis) and PLS-DA, and the standards of differential lipids are fold change >2 or fold change <0.5, P <0.05, and VIP >1. As the result, 70 kinds of lipids are selected as differential lipids, containing 1 Cer, 1 CerG1, 21 PCs, 10 PEs, 8 PIs, 8 PSs, 11 SMs, and 10 TGs, as listed in **Table 2**. These differential lipids are mainly composed with unsaturated long-chain fatty acids and very-long chain fatty acids. Fold change values of 8 TGs, 3 SMs and 1 PI are more than 1, and these 12 differential lipids are TG (16:0e/18:1/18:1), TG(20:0p/16:0/16:0), TG(16:0/14:0/22:6), TG(15:0/18:1/20:5), TG(15:0/18:1/20:5) isomers, TG(18:2/17:1/18:2), TG(18:0e/ 18:1/18:1), TG(18:0/16:0/22:6), SM (d43:4), SM (d44:4), SM (d22:1+hO/18:0), and PI(18:0/20:3) isomers. This result means that contents of these 12 lipids referred are higher in camel milk than that in cow milk.

TG, which is composed of a glycerol main chain and three fatty acid chains, is an important part of lipid nucleus in milk fat globule and plays an important role in metabolism and energy stores (Melissa et al., 2019). Furthermore, number of TG identified from cow milk are more than two times higher than camel milk. Among all lipids identified (**Figure 4A**), TG(16:0/18:1/18:1) shows the highest content in camel

ACCEPTED

milk, which is same with the result of Xiao (2022), while TG(6:0/14:0/16:0) shows the highest content in cow milk.

SM, as a key lipid species in milk fat globule, is important for controlling intestinal microbial interactions and myelin production in the central nervous system (Ghn et al., 2019). Camel milk contains more SM (d43:4), SM (d44:4) and SM (d22:1+hO/18:0) than cow milk, and this is not similar with the analysis with lipids in milk fat globule of camel milk (He et al., 2024). Thus, when compared with camel milk, higher content of SM (d43:4), SM (d44:4) and SM (d22:1+hO/18:0) would featured camel milk.

PI also is an bioactive lipid in milk, and may contribute to the anti-inflammatory and immunoenhancement activity of milk (Xiao, 2022). PI(18:0/20:3) isomers has been reported in Alxa Bactrian camel milk, and camel milk contains more PI(18:0/20:3) isomers than cow milk from Alxa, Inner Mongolia, China (He et al., 2024). This result is same with our study.

Therefore, determination of TG(16:0/18:1/18:1), TG(6:0/14:0/16:0), SM (d43:4), SM (d44:4), SM (d22:1+hO/18:0), and PI(18:0/20:3) isomers could be a potential method for the identification of dairy products adulteration. Now, the qualitative and quantitative analysis of lipid have not been finished completely, which would become useful used in the analysis of food composition and will contribute to the in-depth study of lipid function. It also offer some foundation for the process of camel milk, because during the heating process the oxidative hydrolysis of lipids is one of the important factors affecting the nutrition, quality, and safety of milk and milk products.

ACCEPTED

Hierarchical cluster analysis and analysis of lipid metabolism-related pathways

In order to visualize relationship of these different milk samples and the profile of differential lipids identified in different batches of milk samples, a heat map visualization and hierarchical analysis of the 70 lipids that differed significantly between camel and cow milk samples is shown in Figure 4B. Notably, 6 batches of camel milk clustered into one group, and 6 batches of cow milk clustered into the other group.

Camel, cow, and sheep all are ruminants, and they have different lipid synthesis pathway with non-ruminant animals, referred as acetate and β-Hydroxybutyrate are the principal precursors of fat acid chains with C4-C16 in ruminant animals, while sugar in blood is the principal precursors of fat acids in non-ruminant animals (Bakry et al., 2021). All differential lipid metabolites in camel and cow milk were subjected to enrichment analysis in RaMP library, as showed in **Figure 5** and **Table S1**.

These differential lipids were primarily found to be associated with synthesis of PS, acyl chain remodelling of PS, synthesis of PE, glycerolipids and glycerophospholipids, glycerophospholipid biosynthetic pathway, glycerophos pholipid biosynthesis, phospholipid metabolism, and metabolism of lipids. PC, PS, and PE are all involved in glycerophospholipid metabolism, with glycerophospholipids playing vital roles in cell metabolism, signal transduction, and membrane transport (Liu et al., 2023). According to the results of this study, there still are some differences on the synthesis of fat acids and lipids between camel and cow, especially about TG, PI and SM, and these differences should be analyzed by other omics methods, and no information is given when analyzed according to lipidomics data mainly due to the limitation of database.

ACCEPTED

Conclusions

In conclusion, the non-targeted lipid relative quantitative analysis of Holstein cow milk and Junggar Bactrian camel milk was carried out by UPLC-MS/MS technology, and 669 kinds of lipids are identified in total. In results of PCA, PLS-DA, and OPLS-DA. Six batches of camel milk and six batches of cow milk are separated well, and 70 kinds of differential lipids are selected out, containing 1 Cer, 1 CerG1, 21 PCs, 10 PEs, 8 PIs, 8 PSs, 11 SMs, and 10 TGs. In hierarchical cluster analysis, camel milk samples and cow milk samples also are clustered well. All these results illustrated that there are many different lipids, and camel milk contains more SM (d43:4), SM (d44:4), TG(20:0p/16:0/16:0), TG(16:0/14:0/22:6), TG(15:0/18:1/20:5), TG(15:0/18:1/20:5) isomers, TG(18:2/17:1/18:2), TG(18:0e/18:1/18:1), TG(18:0/16:0/22:6), SM (d22:1+hO/18:0), and PI(18:0/20:3) isomer than cow milk, which can be used as potential biomarker to distinguish camel milk from cow milk. Our study shows the lipid profile of camel milk and cow milk from Xinjiang province of China, as well as their differential lipids, which can provide foundation for the utilization of lipids from camel milk, and provide a potential reference for the camel milk and dairy products adulteration.

References

Ali M. S. G., & Omar M. I. (2001). Fatty acids and lipids of camel milk and colostrum [J]. Int J Food Sci Nutr, 52(3): 183-287. doi.org/10.1080/0963 7480020027000-3 -5.

Bakry I. A., Yang L., Farag M. A., Mohamed A. F., Sameh A. K., Ibrahim K., Ilaria C., Noha I. Z., Jun J., Qingzhe J., Wei W., & Xingguo W. (2021). A comprehensi

ACCEPTED

ve review of the composition, nutritional value, and functional properties of camel milk fat [J]. Foods, 10(9): 2158-2185. doi.org/10.3390/foods10092158.

Bang G., Kim Y. H., Yoon J., Yu Y. J., Chung S., & Kim J. A. (2017). On-Chip lipid extraction using superabsorbent polymers for mass spectrometry [J]. Anal Chem, 89(24), 13365-13373.

Ghn A., Mm B., Mcm B., & Cnb A. (2019). Protective properties of milk sphingomyelin against dysfunctional lipid metabolism, gut dysbiosis, and inflammation [J]. J Nutr Biochem, 73: 108224-108224. doi.org/10.1016/j.jnutbio.2019.108224.

He J, Si R, Wang Y, Ji R, Ming L. (2024). Lipidomic and proteomic profiling identifies the milk fat globule membrane composition of milk from cows and camels [J]. Food Res Int, 179: 113816. doi.org/10.1016/j.foodres.2023.113816.

Ibrahim AB, Wei W, Mohamed AF, Sameh AK, Ibrahim K, Noha IZ, Hanan KM, Jun J, Xingguo W. (2023). How does camel milk fat profile compare with that of human milk fat to serve as a substitute for human milk? [J]. Inter Dairy J, 146: 105738. doi.org/10.1016/j.idairyj.2023.105738.

Li L. (2019). Research on therapeutic effect of fat in camel milk on rheumatoid arthritis [D]. Inner Mongolia Agricultural University.

Lina W., Xiaodong L., Lu L., Hong D. Z., Yu Z., Yu H. C., & Qi P. Z. (2020). Comparative lipidomics analysis of human, bovine and caprine milk by UHPLC-Q-TOF-MS [J]. Food Chem, 310, 125865. doi.org/10.1016/j. foodchem.2019.125865.

Liu Y., Guo X., Wang N., Lu S., Dong J., Qi Z., & Wang Q. (2023). Evaluation of changes in egg yolk lipids during storage based on lipidomics through UPLC-

ACCEPTED

MS/MS [J]. Food Chem, 398: 133931. doi.org/10.1016/j.foodchem.2022.133931.

Lordan, R., & Zabetakis, I. (2017). Invited review: The anti-inflammatory properties of dairy lipids. J Dairy Sci, 100(6): 4197-4212. doi.org/10.3168/ jds.2016-12224.

Maqsood S., Al-Dowaila A., Mudgil P., Kamal H., Jobe B., & Hassan H. M. (2019). Comparative characterization of protein and lipid fractions from camel and cow milk, their fun ctionality, antioxidant and antihypertensive properties upon simulated gastro-intestinal digestion. Food Chem, 279, 328-338. doi.org/10.1016/j.foodchem.2018.12.011.

Melissa R. P., Fidel S. P., Sheher B. M., Jonathon H., & Stephanie M. C. (2019). Lipid omic analysis reveals altered fatty acid metabolism in the liver of the symptomati c niemann–pick, ypе C1 mouse model. Proteomics, 19(18): 1800285. doi.org/10.1002/pmic.201800285.

Miao J., Xiao S., Wang J. (2023). Comparative Study of Camel Milk from Different A reas of Xinjiang Province in China [J]. Food Sci Anim Resour, 43(4): 674-684. doi.org/10.5851/kosfa.2023.e27.

Ming L., Li Y. F., Lyu H., Hosblig, & Yi L. (2023). Chemical Composition and Proteomics of Camel Milk at Different Lactation Stages [J]. Food Science. http://kns.cnki.net/kcms/detail/11.2206.TS.20231215.1713.018.html.

Robert G. J. (2002). The composition of bovine milk lipids [J]. J Dairy Sci, 85(2): 295-

ACCEPTED

391 350. doi.org/10.3168/jds.S0022- 0302(02)74079-4.
392 Sioriki E., Smith T. K., Demopoulos C. A., & Zabetakis I. (2016). Structure and cardi
393 oprotective activities of polar lipids of olive pomace, olive pomace-
394 enriched fish feed and olive pomace fed gilthead sea bream (Sparus aurata). Food
395 Res Int, 83(5): 143–151. doi.org/10.1016/j.foodres.2016.03.015.
396 Siscovick D. S., Barringer T. A., Fretts A. M., Wu J. H. Y., Lichtenstein A. H., Costell
397 o R. B., & Mozaffarian D. (2017). Omega-
398 3 polyunsaturated fatty acid (Fish Oil) supplementation and the prevention of clin
399 ical cardiovascular disease: A science advisory from the american heart associatio
400 n. Circulation, 135(15): e867-e884. doi.org/10.1161/CIR.0000000000000482.
401 Sun Q., Ma J., Campos H., & Hu F. B. (2007). Plasma and erythrocyte biomarkers of
402 dairy fat intake and risk of ischemic heart disease [J]. The Amer J clin Nut, 86(4):
403 929-937. doi.org/10.1093/ajcn/86.4.929.
404 Wang F., Chen M., Luo R., Huang G., Wu X., Zheng N., Zhang Y., Wang J. (2022).
405 Fatty acid profiles of milk from Holstein cows, Jersey cows, buffalos, yaks, humans,
406 goats, camels, and donkeys based on gas chromatography-mass spectrometry [J]. J
407 Dairy Sci, 105(2): 1687-1700. doi.org/10.3168/jds.2021-20750.
408 Wen R. (2023) Study on the variance of lipid and fat-soluble vitamins in non-bovien
409 milks and its fermented milk in WEt China [D]. northwest Agriculture and Forestry
410 University.
411 Xiao Z. Y. (2022). Research on differences in protein, metabolite, and lipid composition
412 of camel milk during lactation based on omics technologies [D]. Inner Mongolia

ACCEPTED

413 Agricultural University.
414 Xu Y. D., Hong H. H., Lin X. Q., Tong T., Zhang J. J., He H. T., Yang L. L., Mao G. F.,
415 Hao R. R., Deng P., Yu Z. P., Pi H. F., Cheng Y., Zhou Z. (2023). Chronic cadmium
416 exposure induces Parkinson-like syndrome by eliciting sphingolipid disturbance
417 and neuroinflammation in the midbrain of C57BL/6J mice [J]. Environmental
418 Pollution, 337: 122606. doi.org/10.1016/j.envpol.2023.122606.
419 Yun W. U., Quan S., Li X. L., Wu X. Y., Wuni M. H., & Yu Y. (2013). Fatty acid comp
420 osition of Alxa bactrian camel hump fat [J]. China Oils and Fats, 38(12): 88-
421 90. doi.org/10.3969/j.issn.1003-7969.2013.12.022.
422

ACCEPTED

Tables and Figures

Table 1. Information of camel milk and cow milk samples collected from different areas

Groups		Place of origin	Purchasing Agency
Camel milk	Camel-1	Midong district of Urumqi city	Milk mixture of 35 camels of a local family of nomads
	Camel-2	Dabancheng district of Urumqi city	Milk mixture of 9 camels of a local family of nomads
	Camel-3	Midong district of Urumqi city	Milk mixture of 11 camels of a local family of nomads
	Camel-4	Changji city of Changji region	Milk mixture of 6 camels of a local family of nomads
	Camel-5	Jeminay county of of Altay region	Milk mixture of 54 camels of a local family of nomads in Wantuo Garden
	Camel-6	Yiwu county of Hami region	Milk mixture of 52 camels of a local family of nomads
Cow milk	Cow-1	Midong district of Urumqi city	Milk mixture of 10 cows of a local family of nomads
	Cow-2	Dabancheng district of Urumqi city	Milk mixture of 13 cows of a local family of nomads
	Cow-3	Fukang city of Changji region	Milk mixture of 6 cows of a local family of nomads
	Cow-4	Changji city of Changji region	Milk mixture of 9 cows of a local family of nomads
	Cow-5	Jeminay county of of Altay region	Milk mixture of 21 cows of a local family of nomads
	Cow-6	Yiwu county of Hami region	Milk mixture of 10 cows of a local family of nomads

ACCEPTED

427 **Table 2. Differential lipids selected from camel milk and cow milk**

Lipid	Fold Change	P value	VIP	Type of lipid
PE(16:0/18:1)	0.25309	0.0025259	1.01970737	PE
PE(16:0/20:4)	0.15995	0.0026412	1.00830031	PE
PS(18:0/16:1)	0.19935	0.0051777	1.116523874	PS
PS(18:0/18:1)	0.32328	0.0033796	1.044887608	PS
PC(18:0/16:0)	0.26896	0.0031288	1.063964627	PC
PS(18:0/20:3)	0.22012	0.0020539	1.006795602	PS
SM(d39:1)	0.19196	0.0026884	1.023107395	SM
PI(16:0/18:1)	0.22664	0.0035707	1.063223089	PI
SM(d32:1)	0.48353	0.0093506	1.128564204	SM
SM(d35:4)	0.49487	0.020952	1.320660195	SM
SM(d36:3)	0.28469	0.0020427	1.060805023	SM
SM(d36:2)	0.48188	0.045415	1.180984105	SM
PC(32:2) isomers	0.40786	0.0039475	1.079901437	PC
PC(32:1) isomers	0.33675	0.0063529	1.061210879	PC
PC(32:2)	0.44533	0.0075071	1.166917013	PC
PC(32:1)	0.41543	0.004971	1.145357814	PC
PE(18:0p/20:3)	0.45546	0.033668	1.260402519	PE
PC(34:2)	0.36726	0.0054166	1.194271727	PC
SM(d38:1)	0.36308	0.0022285	1.006439614	SM
PC(33:0)	0.44202	0.012582	1.193822157	PC
PC(35:1)	0.48624	0.0075943	1.186496428	PC
PC(35:0)	0.48198	0.014578	1.2305842	PC
TG(15:0/14:0/16:1)	0.3097	0.008346	1.036468064	TG
PC(36:3)	0.37843	0.0034672	1.135846161	PC
PC(36:3) isomers	0.33629	0.0076136	1.14531328	PC
CerG1(d38:1+hO)	0.10257	0.0081079	1.015455237	CerG1
TG(15:0/14:0/18:3)	0.40762	0.0054697	1.106992447	TG
PC(38:5)	0.31377	0.005916	1.152986436	PC
PC(38:4)	0.46926	0.02403	1.345120674	PC
SM(d43:4)	3.5167	0.017268	1.309258008	SM
PC(38:5) isomers	0.40066	0.036169	1.326866495	PC
SM(d44:4)	4.5197	0.0058818	1.165269475	SM
TG(16:0e/18:1/18:1)	6.4364	0.0045916	1.164245233	TG
PI(36:2)	0.43393	0.011173	1.147317794	PI
TG(20:0p/16:0/16:0)	6.1591	0.0045188	1.145231807	TG
TG(16:0/14:0/22:6)	2.1428	0.046183	1.393556905	TG
PI(36:2) isomers 1	0.42886	0.0079825	1.149268005	PI
TG(15:0/18:1/20:5)	2.4988	0.037097	1.38853673	TG
TG(15:0/18:1/20:5) isomers	2.7951	0.03552	1.130337837	TG
TG(18:2/17:1/18:2)	2.4223	0.041155	1.387784722	TG
PI(36:2) isomers 2	0.40303	0.0055327	1.187322474	PI
TG(18:0e/18:1/18:1)	7.1976	0.013502	1.290160035	TG
TG(18:0/16:0/22:6)	2.8339	0.041309	1.252979471	TG
Cer(d16:1/22:0)	0.4285	0.0046595	1.019762745	Cer
PE(16:0/16:1)	0.36871	0.0063953	1.146101909	PE
PE(15:0/18:1)	0.32294	0.0031315	1.013861544	PE
PE(16:1/18:1)	0.27306	0.0046592	1.070492673	PE
PE(17:0/18:2)	0.43123	0.0057484	1.110006911	PE

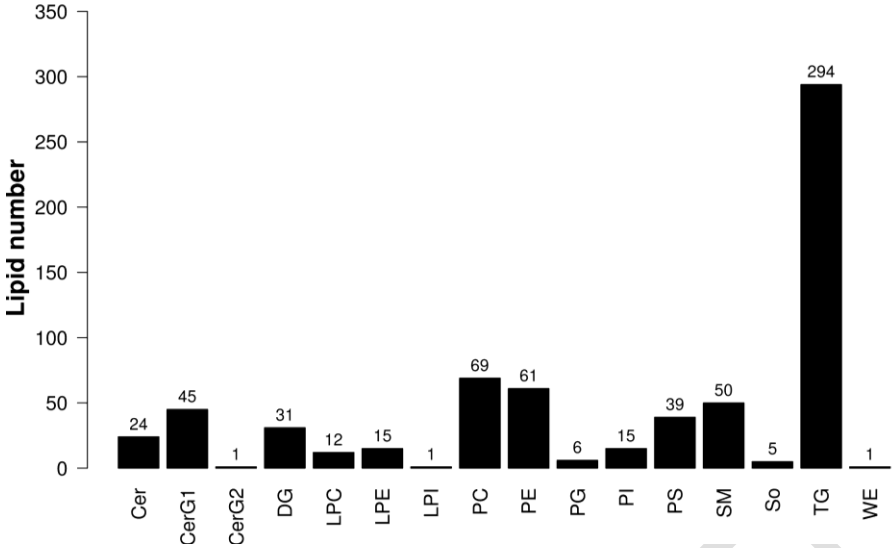
ACCEPTED

SM(d33:1)	0.48583	0.0090071	1.163648046	SM
PE(18:1/18:2)	0.35939	0.0062357	1.012923978	PE
PS(34:3)	0.38212	0.0058944	1.129656914	PS
PE(18:1/20:3)	0.17809	0.020747	1.070505286	PE
PE(20:1/18:1)	0.38611	0.035727	1.25359712	PE
PC(14:0/18:2)	0.45432	0.011393	1.057459718	PC
PC(16:0/16:1)	0.41245	0.0064837	1.069792082	PC
PS(18:2/18:2)	0.32335	0.0028439	1.02971887	PS
PC(15:0/18:1)	0.36541	0.0034178	1.006341343	PC
PS(37:4)	0.3765	0.0048371	1.039158331	PS
PS(37:3)	0.4909	0.024573	1.317344351	PS
PC(16:0/18:2)	0.44868	0.0059025	1.104107353	PC
PS(20:1/18:1)	0.24173	0.01809	1.100185404	PS
PC(17:0/18:2)	0.4845	0.024175	1.186291059	PC
PI(16:0/18:2)	0.30176	0.0038736	1.070089487	PI
SM(d22:1+hO/18:0)	2.3738	0.0048732	1.051158985	SM
PC(18:1/20:4)	0.38436	0.013085	1.10206223	PC
PC(18:0/20:3)	0.45775	0.025288	1.17596455	PC
PI(18:1/18:1)	0.46048	0.01054	1.114126653	PI
PI(18:0/20:3)	0.47224	0.017549	1.016683377	PI
PI(18:0/20:3) isomers	2.4697	0.013091	1.028695068	PI
SM(d31:1)	0.45072	0.0098512	1.17322489	SM

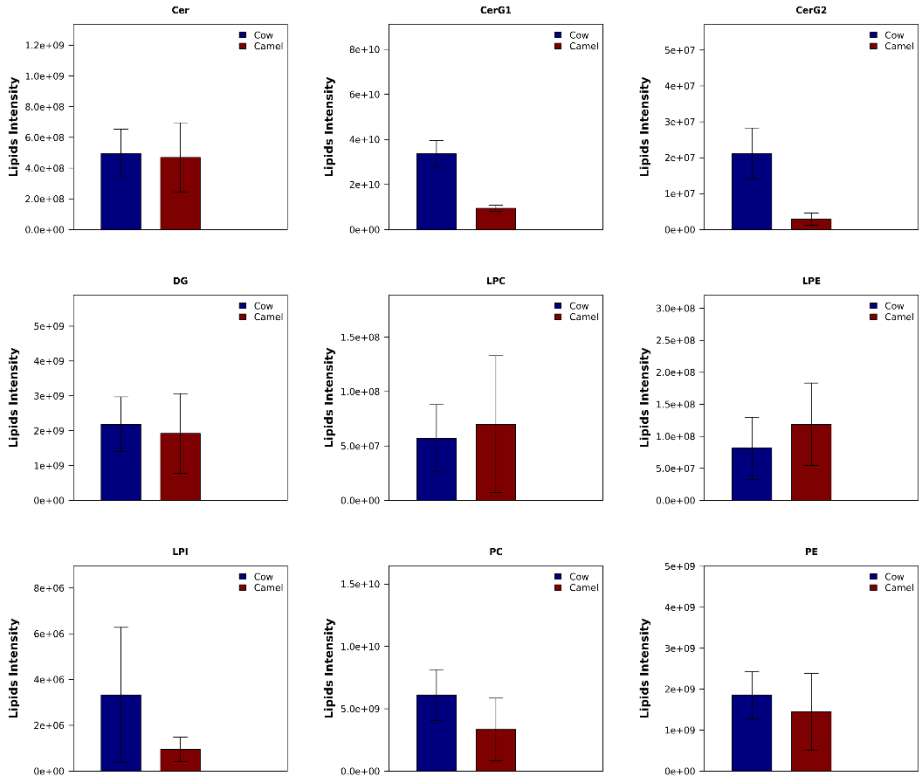
428

ACCEPTED

(A)


Lipid number
350
300
250
200
150
100
50
0
24
45
1
31
12
15
1
69
61
6
15
39
50
5
294
1
Cer
CerG1
CerG2
DG
LPC
LPE
LPI
PC
PE
PG
PI
PS
SM
So
TG
WE

(B)


Cer
CerG1
CerG2
DG
LPC
LPE
LPI
PC
PE
Cow
Camel
Lipids Intensity

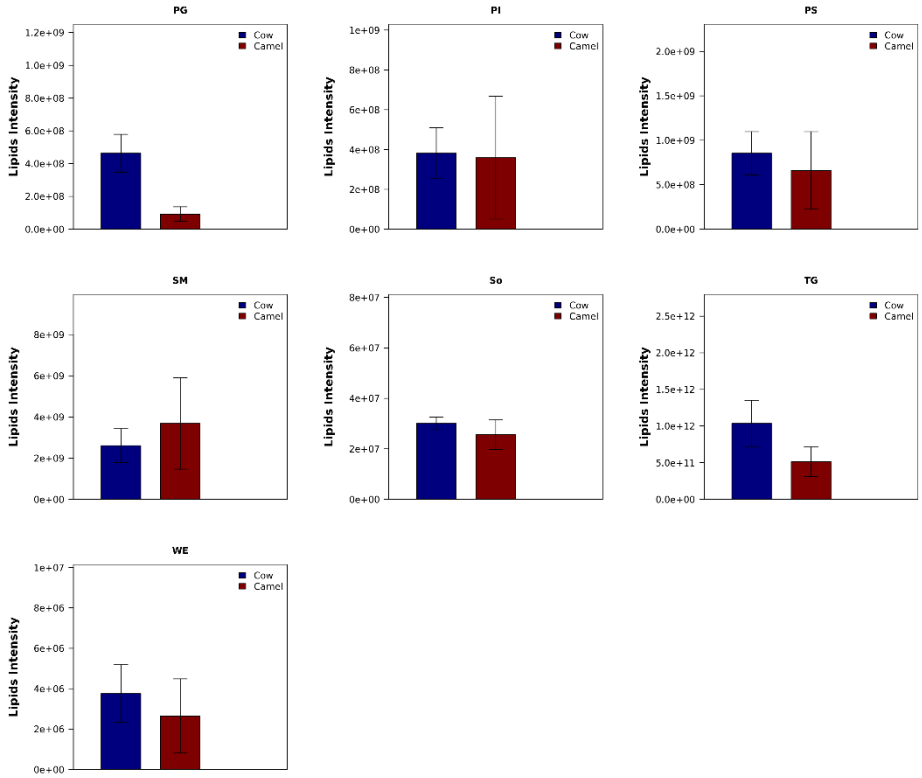


Figure 1 Lipids identified from camel milk and cow milk. (A) Numbers and classes of lipids identified in camel milk and cow milk, (B) Contents of lipids in 16 classes identified from camel milk and cow milk. (letters a and b mean show significant difference at $p<0.05$ level, Cers for ceramides, CerG1s for glycosphingolipids, CerG2s for diglucose ceramide, DGs for diacylglycerides, LPCs for lysophosphatidylcholines, LPEs for lysophosphatidy-lethanolamine, LPIs for lysophosphatidylinositol, PC for phosphatidylcholines, PE for phosphatidylethanolamines, PG for phosphatidylglycerols, PI for phosphatidylinositol, PS for phosphatidylserines, SM for sphingomyelins, So for sphingosines, TG for triacylglyceri-des, WE for wax ester)

ACCEPTED

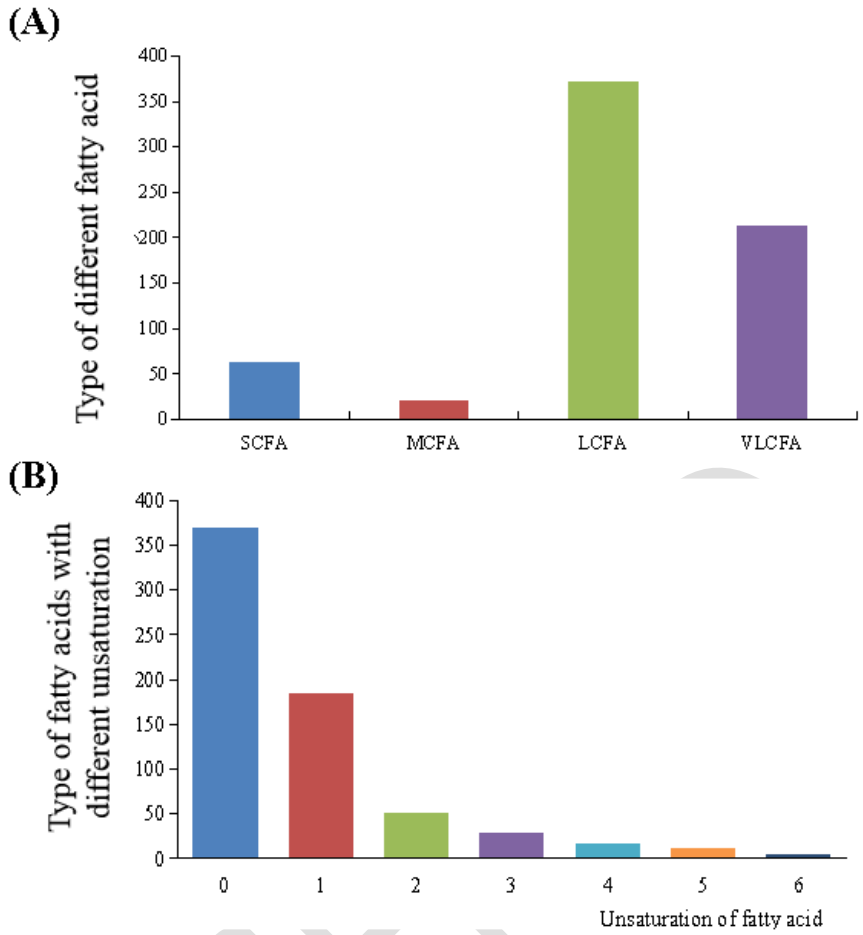
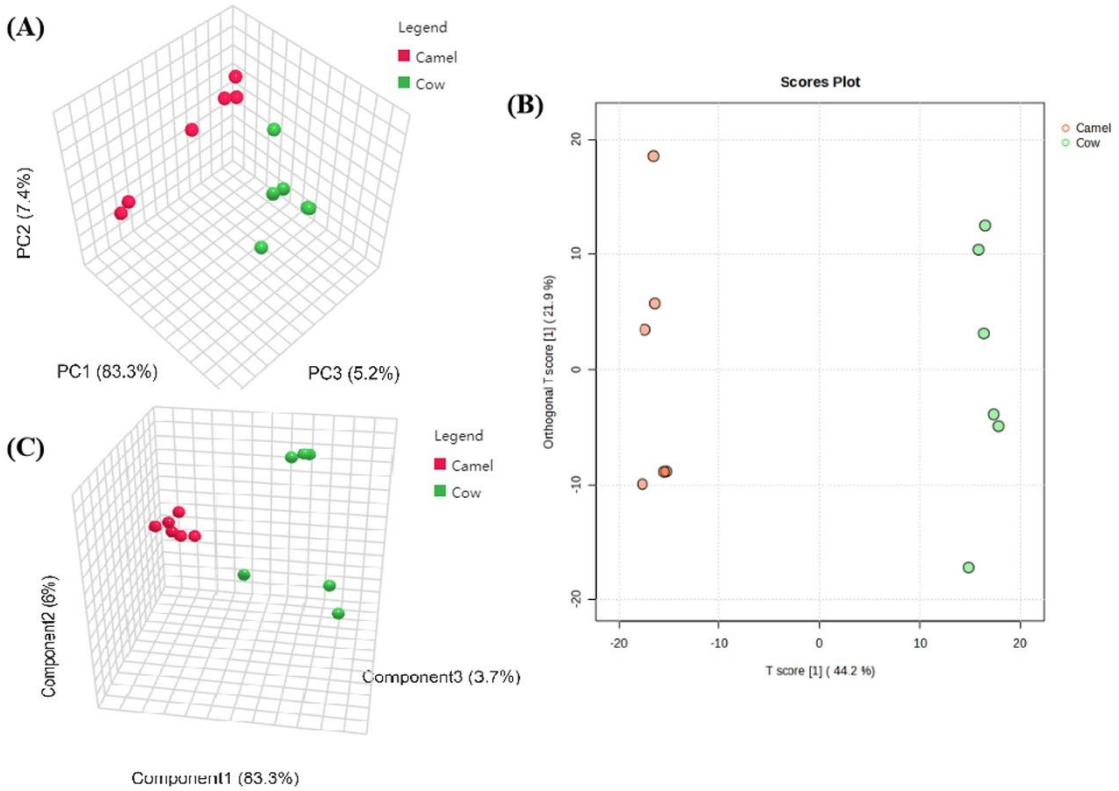


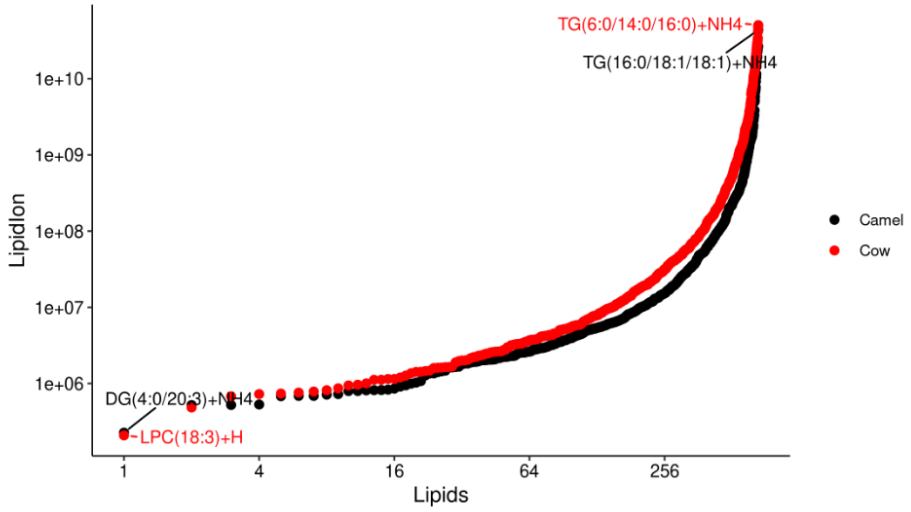
Figure 2 Fatty acid in lipids of camel milk and cow milk. (A) Type of different fatty acids, (B) Types of fatty acids with different unsaturation. (SCFA for short-chain fatty acid, MCFA for medium-chain fatty acid, LCFA for long-chain fatty acids, and VLCFA for very-long chain fatty acid)



448
449 **Figure 3** Multivariate statistical analysis of lipomics in camel milk and cow milk (A: PCA score
450 plot, B: OPLS-DA score plot, C: PLS-DA score plot. t[1] represents the principal component 1,
451 t[2] represents the principal component 2, and the ellipse represents the 95% confidence interval.
452 The dots of the same shape represent the biological repetitions in the group, and the distribution of
453 the dots reflects the degree of difference between and within groups.)

ACCEPT

(A)



(B)

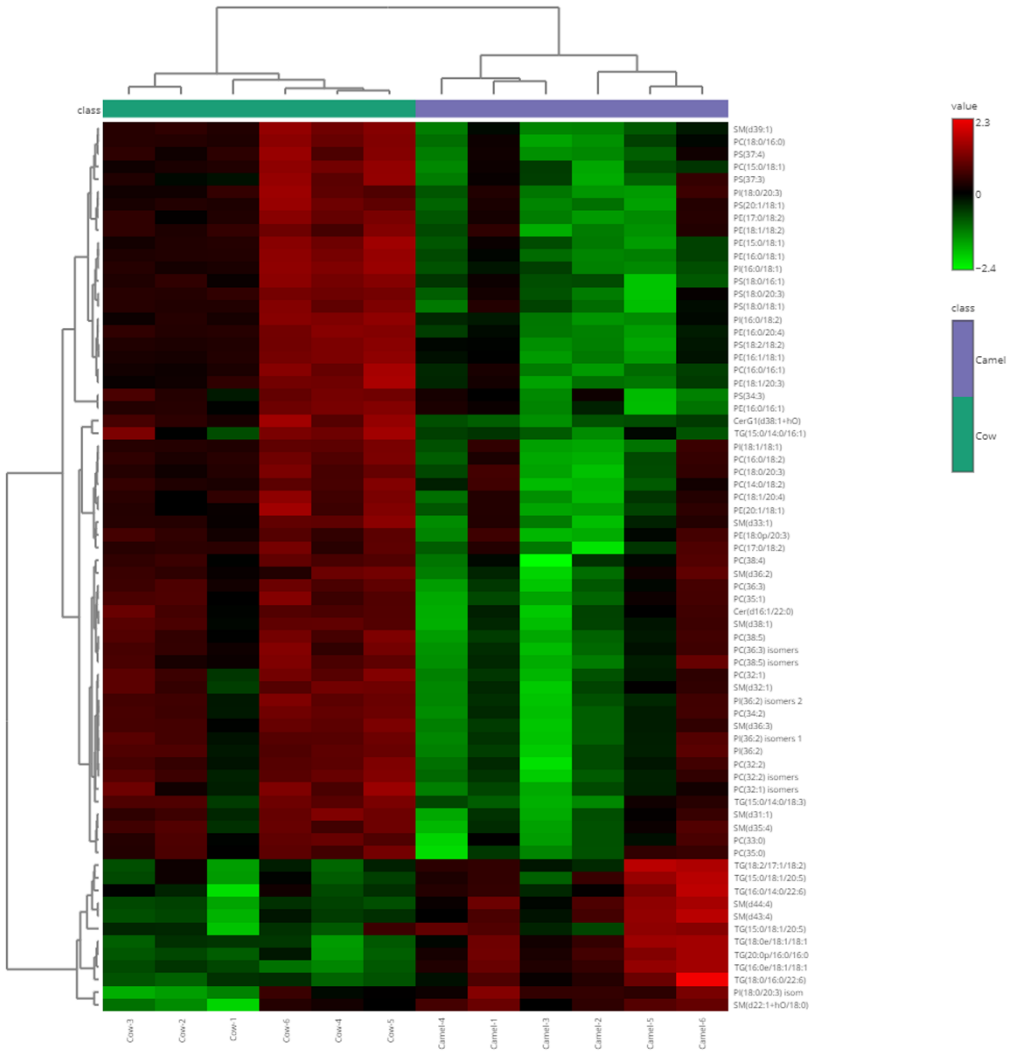


Figure 4 Differential lipids in camel milk and cow milk. (A) Dynamic distribution map of lipids in camel milk and cow milk, (B) Hierarchical clustering heatmap of milk samples and differential lipids.

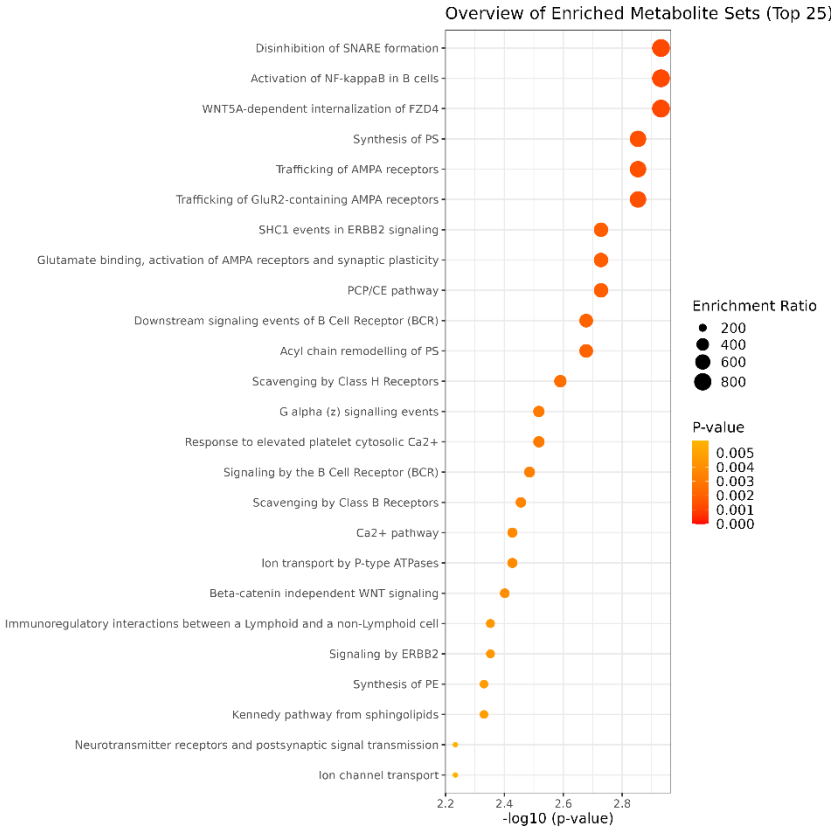
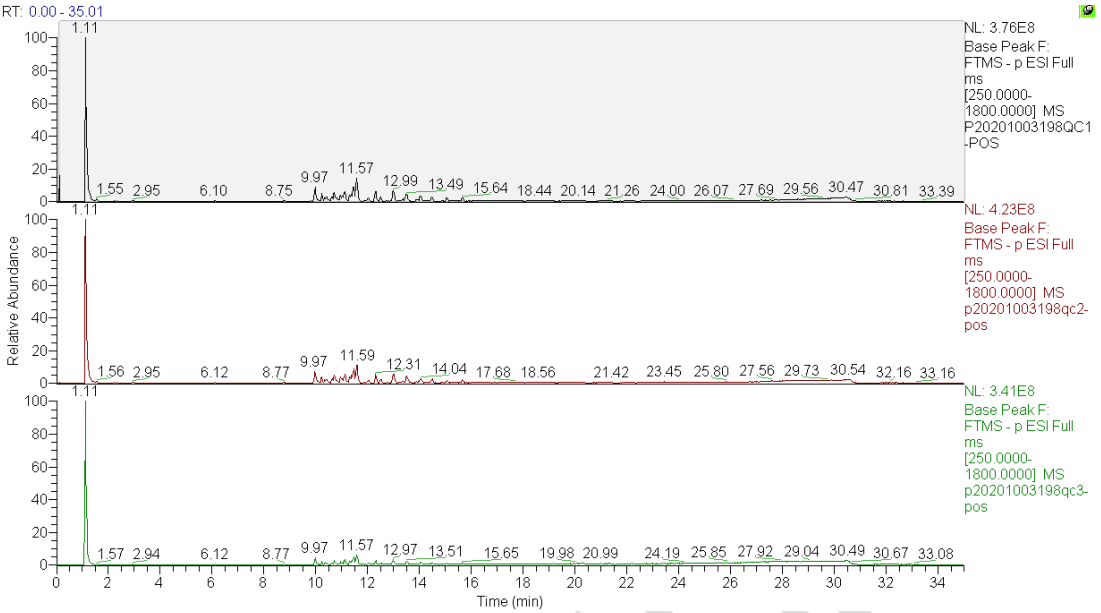


Figure 5 Enrichment analysis of significant lipid biosynthetic pathways

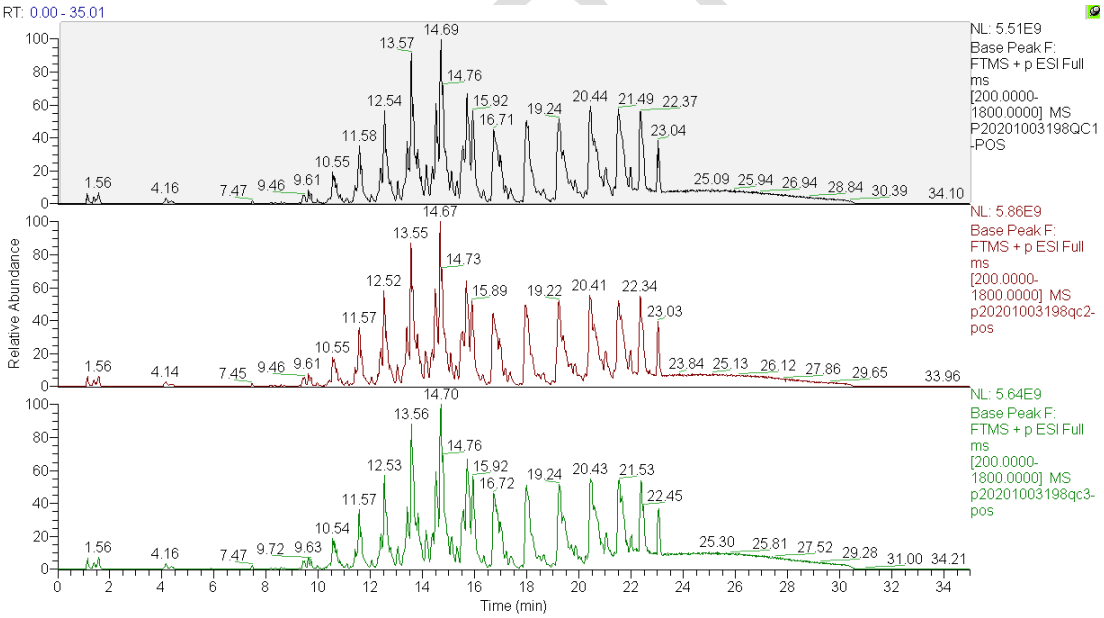
ACCEPTED

Supplementary material

Fig. S1 TIC spectrograms in Positive mode of three QC samples

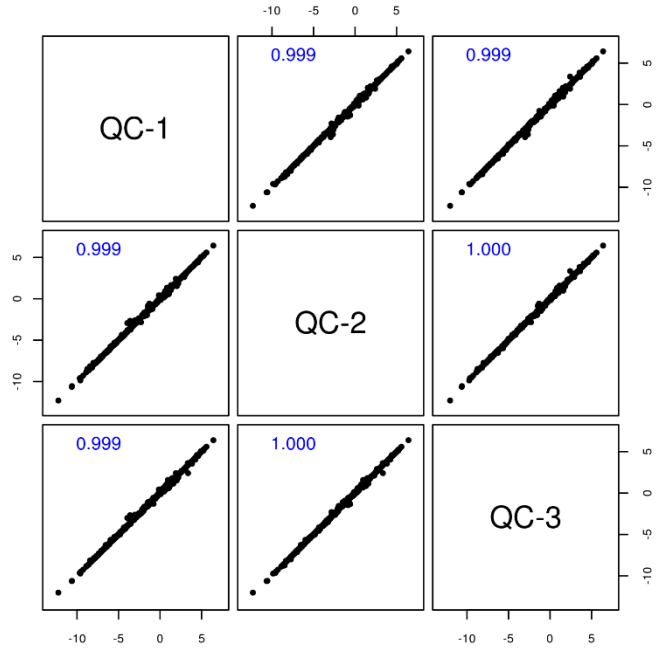


(A) TIC spectrograms in Positive mode of three QC samples



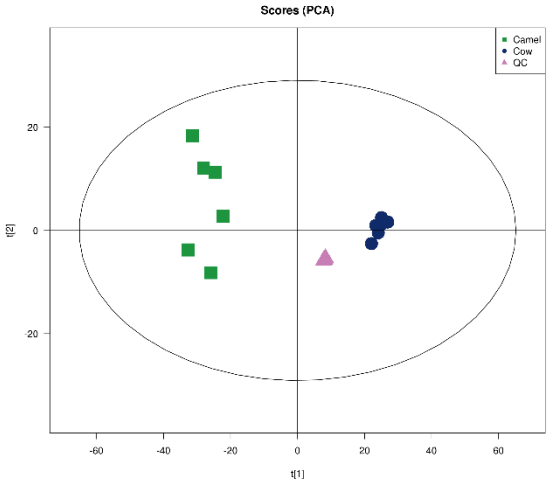
(B) TIC spectrograms in Negative mode of three QC samples

Fig. S2 Correlation coefficients of three batches of QC samples



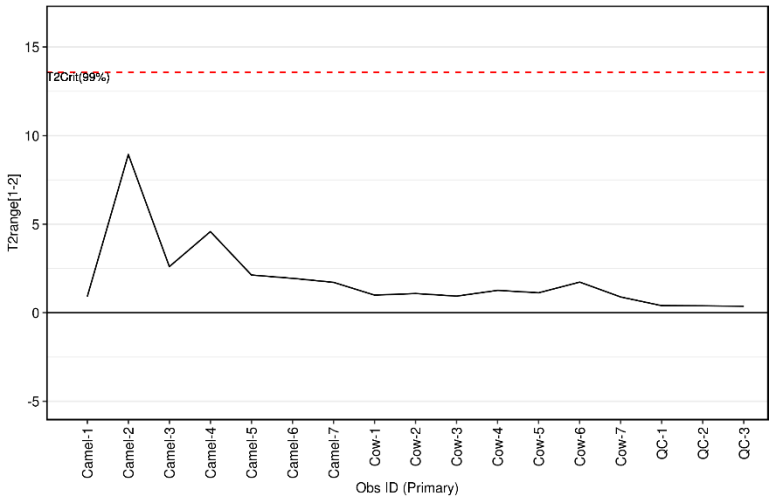
ACCEPTED

Fig. S3 PCA result of three bateches of QC samples



ACCEPTED

Fig. S4 Hotelling T2 test of QC samples, camel milk samples, and cow milk samples



ACCEPTED

Table S1 Result from Over Representation Analysis of Enrichment Analysis

	total	expected	hits	Raw p	Holm p	FDR
Disinhibition of SNARE formation	5	0.00	1	1.17E-03	1.00E+00	6.34E-01
Activation of NF-kappaB in B cells	5	0.00	1	1.17E-03	1.00E+00	6.34E-01
WNT5A-dependent internalization of FZD4	5	0.00	1	1.17E-03	1.00E+00	6.34E-01
Synthesis of PS	6	0.00	1	1.40E-03	1.00E+00	6.34E-01
Trafficking of AMPA receptors	6	0.00	1	1.40E-03	1.00E+00	6.34E-01
Trafficking of GluR2-containing AMPA receptors	6	0.00	1	1.40E-03	1.00E+00	6.34E-01
SHC1 events in ERBB2 signaling	8	0.00	1	1.87E-03	1.00E+00	6.34E-01
Glutamate binding, activation of AMPA receptors and synaptic plasticity	8	0.00	1	1.87E-03	1.00E+00	6.34E-01
PCP/CE pathway	8	0.00	1	1.87E-03	1.00E+00	6.34E-01
Downstream signaling events of B Cell Receptor (BCR)	9	0.00	1	2.10E-03	1.00E+00	6.34E-01
Acyl chain remodelling of PS	9	0.00	1	2.10E-03	1.00E+00	6.34E-01
Scavenging by Class H Receptors	11	0.00	1	2.57E-03	1.00E+00	6.74E-01
G alpha (z) signalling events	13	0.00	1	3.04E-03	1.00E+00	6.74E-01
Response to elevated platelet cytosolic Ca2+	13	0.00	1	3.04E-03	1.00E+00	6.74E-01
Signaling by the B Cell Receptor (BCR)	14	0.00	1	3.27E-03	1.00E+00	6.74E-01
Scavenging by Class B Receptors	15	0.00	1	3.50E-03	1.00E+00	6.74E-01
Ca2+ pathway	16	0.00	1	3.74E-03	1.00E+00	6.74E-01
Ion transport by P-type ATPases	16	0.00	1	3.74E-03	1.00E+00	6.74E-01
Beta-catenin independent WNT signaling	17	0.00	1	3.97E-03	1.00E+00	6.74E-01
Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	19	0.00	1	4.44E-03	1.00E+00	6.74E-01
Signaling by ERBB2	19	0.00	1	4.44E-03	1.00E+00	6.74E-01
Synthesis of PE	20	0.00	1	4.67E-03	1.00E+00	6.74E-01
Kennedy pathway from sphingolipids	20	0.00	1	4.67E-03	1.00E+00	6.74E-01
Neurotransmitter receptors and postsynaptic signal transmission	25	0.01	1	5.84E-03	1.00E+00	7.45E-01
Ion channel transport	25	0.01	1	5.84E-03	1.00E+00	7.45E-01
Signaling by WNT	25	0.01	1	5.84E-03	1.00E+00	7.45E-01
Glycerolipids and glycerophospholipids	28	0.01	1	6.54E-03	1.00E+00	8.03E-01
Scavenging by Class A Receptors	30	0.01	1	7.01E-03	1.00E+00	8.30E-01
Platelet activation, signaling and aggregation	32	0.01	1	7.47E-03	1.00E+00	8.55E-01
Binding and Uptake of Ligands by Scavenger Receptors	39	0.01	1	9.11E-03	1.00E+00	1.00E+00
Adaptive Immune System	53	0.01	1	1.24E-02	1.00E+00	1.00E+00
Glycerophospholipid biosynthetic pathway	54	0.01	1	1.26E-02	1.00E+00	1.00E+00
Signaling by Receptor Tyrosine Kinases	62	0.01	1	1.45E-02	1.00E+00	1.00E+00
Vesicle-mediated transport	63	0.01	1	1.47E-02	1.00E+00	1.00E+00
Transmission across Chemical Synapses	77	0.02	1	1.80E-02	1.00E+00	1.00E+00
Neuronal System	77	0.02	1	1.80E-02	1.00E+00	1.00E+00
Hemostasis	82	0.02	1	1.92E-02	1.00E+00	1.00E+00
Glycerophospholipid biosynthesis	97	0.02	1	2.27E-02	1.00E+00	1.00E+00
Phospholipid metabolism	105	0.02	1	2.45E-02	1.00E+00	1.00E+00
Immune System	155	0.04	1	3.62E-02	1.00E+00	1.00E+00
GPCR downstream signalling	156	0.04	1	3.64E-02	1.00E+00	1.00E+00
Transport of small molecules	208	0.05	1	4.86E-02	1.00E+00	1.00E+00
Signaling by GPCR	236	0.06	1	5.51E-02	1.00E+00	1.00E+00
Signal Transduction	345	0.08	1	8.06E-02	1.00E+00	1.00E+00
Metabolism of lipids	608	0.14	1	1.42E-01	1.00E+00	1.00E+00
Metabolism	1370	0.32	1	3.20E-01	1.00E+00	1.00E+00