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Lipidomics analysis for identifying the geographical origin and lactation stage of goat milk

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ABSTRACT

Goat milk samples of three lactation stages (colostrum, mature and late milk) were collected from three farms and analyzed with an untargeted method based on UPLC-Q-Exactive Orbitrap Mass Spectrometry and multivariate statistics. A total of 14 lipid subclasses and 756 lipid molecules were identified in samples. Five lipid subclasses and 51 lipid molecules in milk were significantly different among different geographical origins. Two lipid subclasses and 26 lipid molecules were significantly different among different lactation stages. Combined with the partial least squares discriminant analysis results of lipid molecules with a VIP value (Variable Importance in the projection) higher than 1, totally 38 and 19 lipid molecules could be used as potential indicators to identify geographical origins and lactation stages, respectively. Based on six and five selected molecules, the correct rates of discrimination models for geographical origin and lactation stage respectively reached 100% and 96%.

1. Introduction

Recent years, goat milk is of particular interest due to its benefits to human beings, especially to infants and elder people. The proteins and fats in goat milk is more digestible than those in cow milk produced under similar conditions. In addition, goat milk was reported to contain the higher contents of short- and medium-chain fatty acids (FA), USFA, ω-6 FA, ω-3 FA, EPA and DHA than cow milk (Ceballos et al., 2009; Li et al., 2017). In order to better utilize the fat nutrient in goat milk, it is necessary to comprehensively investigate their composition and variations.

The geographical origin of a food is widely concerned among consumers. Foods produced in good producing areas (such as protected designation of origin and protected geographical indication) tend to have higher prices and are prone to be faked in the marketplace. Recent years, many techniques had been developed to trace the geographical origins of milk products, such as stable isotopes (Crittenden et al., 2007; Scampicchio et al., 2012), multi-elements (Hoffman et al., 2018), infrared spectroscopy (Lerma-Garcia, Gori, Cerretani, Simo-Alfonso, & Caboni, 2010; Bassbasi, De Luca, Ioele, Oussama, & Ragno, 2014), and the combination of stable isotopic ratios and elements (Griboff, Baroni, Horacek, Wunderlin, & Monferran, 2019; Liu, Jiao, et al., 2019, Liu, Zhao, et al., 2019). These methods have the good discrimination capability, but the data obtained by the above methods are not related to the quality of food itself. The physical structure and chemical composition of lipids could affect the nutritional value, sensory quality and the processing characteristics of foods (Chen et al., 2017), then both the geographical origin and food quality can be identified by lipid profile analysis.

The lactation stage significantly affects the milk composition, but the changing trend of the fat content was not consistent with the whole lactation period (Assan, 2014). It was reported that the fat content was the highest at the late lactation stage, followed by early lactation stage and middle lactation stage, because the fat content was negatively correlated with milk yield (Baker, 2007). It was reported that the fat content gradually decreased in the lactation period because de novo lipogenesis was usually more active after peak lactation time (Bouattour, Casals, Albanell, Such, & Caja, 2008). Most of studies focused on the effect of lactation on the fatty acid profile (Sinanoglou et al., 2015; Mele et al., 2016; Qi et al., 2018) and fat content (Yilmaz, Cak, & Bolacali, 2011; Mestawet et al., 2012) of raw milk. Most of the above studies focused on the limited classes of lipid, but few studies

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investigated the comprehensive lipid profiles of milk.

In order to better understand the physical structure and chemical composition of lipids in milk, it is necessary to develop a powerful detection technique with higher resolution, sensitivity and accuracy. Lipidomics is an important component of metabolomics and can accurately and thoroughly describe the lipid profiles, structures, functions, interactions and dynamics within a cell or tissue (Chen et al., 2017; Liu, Jiao, et al., 2019, Liu, Zhao, et al., 2019). Lipidomics has been applied in food science and safety research for many purposes, including animal species and breed discrimination (Li et al., 2017; Mi et al., 2018), processing effect (Navarro-Reig, Tauler, Iriondo-Frias, & Jaumot, 2019), storage changes (Wang and Zhang, 2011) and geographical traceability (Mi et al., 2019), but until now, this method has been seldom used to identify the geographical origin or lactation stage of goat milk, while the lactation stage identification is very important especially for the colostrum.

In this study, non-target lipidomics of goat milk in China was investigated. The lipid profiles of milk were analyzed according to different lactation stages and geographical origins. Based on statistical analysis, the effective biomarkers were selected to establish corresponding discrimination models. This work will provide more comprehensive lipid information for discriminating goat milk of lactation stages or from different regions.

2. Materials and methods

2.1. Sample collection

Thirty mature goat milk samples (20–210 days) were collected from Yunnan (10), Shaanxi (8) and Shandong Provinces (12) in autumn of 2017. In addition, 10 colostrum samples (1–10 days) and 10 goat milk samples of late stage (> 210 days) were collected from the same farm in Shaanxi Province. At the same time, feed samples were also collected and the feed formula of each farm was recorded. Feed samples were stored in ice during shipment. All the milk samples were shipped with dry ice as a refrigerant and then stored in −80 °C until analysis. The data of goat milk samples and feed samples are listed in Table 1, and the fatty acid results of feed sample are listed in Table S1.

2.2. Sample pretreatment

Lipid extraction was performed according to the method by Li et al. (2017) with slight modifications. Firstly, 1 mL of Milli-Q water and 3 mL of the mixture of $CHCl_3$: MeOH (2:1, v/v) were added in 0.2 mL of goat milk. The mixture was shaken well for 10 min at 4 °C and then centrifuged for 15 min (2000 rpm, 4 °C). The lower organic phase was pipetted onto a new 1.5-mL frozen tube, dried in nitrogen circulation, then re-dissolved in 200 μL of the mixture of $CHCl_3$: MeOH (2:1, v/v) for further analysis.

2.3. UPLC-MS data acquisition

The data acquisition conditions in the report by Tang et al. (2016) were adopted. In HPLC methods, reverse phase chromatography Cortecs C18 column (2.1 × 100 mm, Waters) was used in the positive mode and XSelect CSH C18 column (2.1 × 100 mm, Waters) was used in the negative mode. Firstly, ammonium acetate (0.77 g) was dissolved in 400 mL of HPLC-grade water and then 600 mL of HPLC-grade acetonitrile was added to prepare the mobile phase A. Then, 100 mL of acetonitrile was added into 900 mL of isopropanol to obtain the mobile phase B. The gradient elution procedure was programmed below: 0 min, 37% B; 1 min, 37% B; 4 min, 45% B; 5 min, 52% B; 8 min, 58% B; 11 min, 66% B; 14 min, 70% B; 18 min, 75% B; 20 min, 98% B; 22 min, 98% B; 22.1 min 37% B; 25 min, 37% B.

The MS analysis of lipids was carried out on Q Exactive Orbitrap (Thermo, CA) under the conditions: spray voltage, 2.8 kV for negative ion mode and 3.2 kV for positive ion mode; aux gas flow rate (arb), 10; capillary temperature, 320 °C; mass range (m/z), 200–2000 for negative ion mode and 240–2000 for positive ion mode.

2.4. Statistical analysis

The significances of the statistical differences of potential markers among various regions or various lactation stages were analyzed by one-way analysis of variance (ANOVA) using SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA) after normalization. Principal component analysis (PCA), partial least squares-discriminate analysis (PLS-DA) and linear discrimination analysis (LDA) were conducted by MetaboAnalyst 4.0 online.

3. Results

3.1. Lipid classes of goat milk samples among different groups

Totally, 5 species of cardiolipin (CL), 45 species of ceramide (Cer), 17 species of lysophosphatidylcholine (LPC), 4 species of lysophosphatidylethanolamine (LPE), 36 species of glycerophosphocholin (PC), 80 species of glycerophosphoethanolamine (PE), 9 species of phosphatidylglycerol (PG), 22 species of phosphatidylinositol (PI), 2 species of acyl carnitine (AcCa), 55 species of simple Glc series (HexCer), 56 species of sphingomyelin (SM), 17 species of fatty acid (FA), 15 species of diacylglycerol (DG) and 416 species of triacylglycerol (TG) were identified in the above analysis.

We calculated the sum of each lipid class and analyzed the difference among different groups. Cer and PC in mature goat milk showed the significant difference among different geographical origins ($P < 0.05$) and PI, SM and FA showed extremely significant differences among different geographical origins ($P < 0.01$) (Fig. 1A). The abundances of Cer, PC and SM in goat milk samples from Yunnan were significantly higher than those in milk samples from Shaanxi and Shandong, whereas the abundances of PI and FA in milk samples from Yunnan were extremely significantly lower than those in milk samples from other two provinces. Among different lactation stages, the abundance of PG showed significant differences ($P < 0.05$) and PG in mature milk samples was much higher than those in colostrum and late milk samples. In addition, TG also showed extremely significant differences among different stages ($P < 0.01$) since TG gradually decreased in the lactation period (Fig. 1B).

Table 1
Information on locations, feed conditions and fat content of goat milk samples.

Farms	Samples	Latitude, Longitude	Altitude (m)	Roughage	Concentrate	Fat (%)
Luliang, Yunnan	mature milk	24°51′32″N, 103°30′47″E	1917	maize silage, grass, radish	maize, distiller's grains	2.71 ± 0.39
Yangling, Shaanxi	colostrum mature milk late milk	34°15′02″N, 108°02′16″E	434	grass	maize, soybean meal, wheat bran, rapeseed meal	3.14 ± 1.01
Jimo, Shandong	mature milk	36°27′53″N, 120°17′37″E	21	maize stalks, grass	maize, soybean meal	2.12 ± 0.57

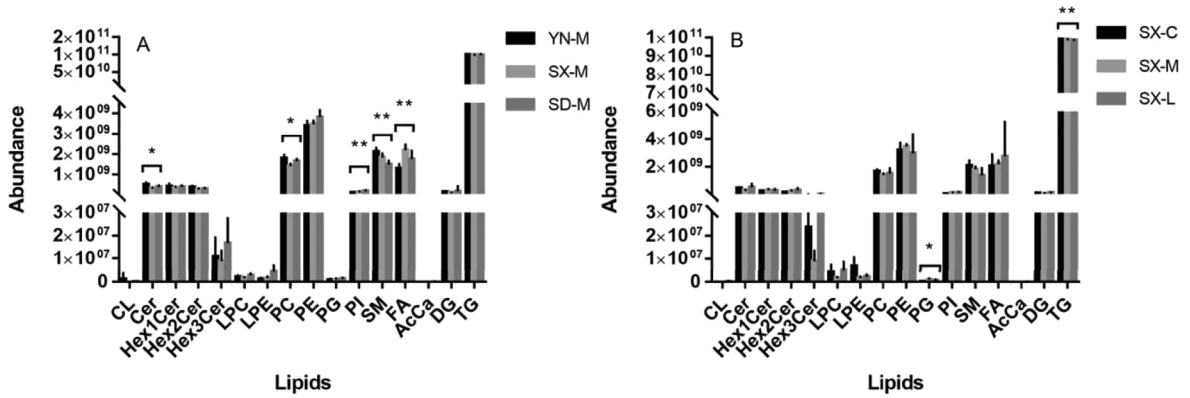

Fig. 1. The abundance of lipid classes among different geographical origins (A) or different lactation stages (B).

3.2. Lipid profiles of goat milk among different geographical origins

Firstly, PCA analysis was performed. The cumulative contribution rate of the first five principal components was 59.6%. Scatter plot was plotted with the first two principal components. Milk samples from Shaanxi could be completely distinguished from milk samples from the other two provinces. There were some overlaps between Shandong and Yunnan samples (Fig. 2A). Secondly, the PLS-DA was performed to discriminate goat milk samples of different geographical origins. The PLS-DA score plot showed that good separation was obtained (Fig. 2B). Based on the VIP (Variable Importance in the projection) values from the PLS-DA model, 259 individual lipid species with VIP > 1 were selected as potential markers for the discrimination of goat milk samples from three provinces: Shaanxi, Shandong and Yunnan. In addition, the significantly different lipid profiles between the three geographical origins were obtained by a one-way ANOVA. The results showed that 51 lipid compounds were significant different among the milk samples from different regions ($P < 0.05$).

The lipids those both satisfied VIP score greater than 1 and ANOVA *p*-value smaller than 0.05 were considered to be potentially significant for the separation of the three different regions. In total, 38 lipids showed significant differences among different geographical origins (Table 2).

3.3. Lipid profiles of goat milk among different lactation stages

We also conducted PCA, PLS-DA and one-way ANOVA to analyze the data of goat milk samples of different lactation stages. According to PCA analysis results, the cumulative contribution rate of the first five principal components was 65%. We used the first three principle components to generate the 3-D scatter plot, the goat milk samples of different lactation stages could be completely separated with each other (Fig. 3A). Fig. 3B shows the PLS-DA score plot based on the classification of goat milk from different lactation stages, there were 251 lipid compounds whose VIP scores were higher than 1. One-way ANOVA was performed to figure out the lipid compounds which were significantly different among different lactation stages ($P < 0.05$), 26 lipid compounds were found to be significantly different in goat milk samples of different lactation stages.

We used the same criterion to find the potential markers for the separation of the goat milk samples of three different lactation stages. The final results showed that 19 lipid compounds (VIP > 1 and $P < 0.05$) could be selected as potential markers for discriminating goat milk samples of different lactation stages (Table 3).

3.4. Establishment of discrimination models

In order to establish discrimination models for goat milk according to geographical origins and lactation stages, the stepwise canonical discriminant analysis was performed with lipidomics data. Two functions were selected and could explain 100% of the variance. Function 1 was mainly obtained by PI(18:1_18:1)-H, TG(11:0_18:1_18:2)+NH_4 and TG(16:1_18:1_18:2)+NH_4, and Function 2 was mainly obtained by TG(6:0_10:0_17:1)+NH_4, TG(10:0_18:2_20:5)+H and TG(16:1_10:0_10:0)+NH_4. Both overall and cross-validation correct classification rate were

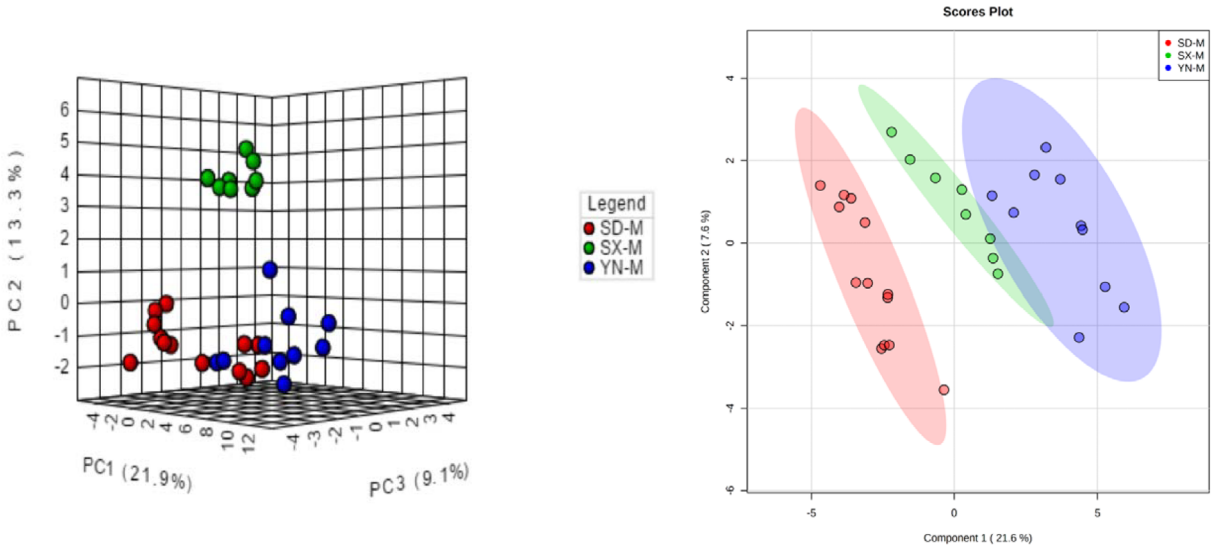

Fig. 2. PCA (A) and PLS-DA (B) of the lipidomic data obtained from the Shandong, Shaanxi and Yunnan mature goat milk samples.

Table 2
The potential lipid makers for discrimination of goat milk geographical origin.

Lipid	Formula	Retention time (min)	Lipid Category	VIP score	P value	Yunnan ($\times 10^6$)	Shaanxi ($\times 10^6$)	Shandong ($\times 10^6$)
Cer(d19:1_24:1) + CH_3COO	C_{45} H_{86} O_5 N_1	21.01	Sphingolipids	1.27	< 0.05	2.52 ± 1.06a	0.45 ± 0.10c	1.32 ± 0.38b
Cer(d18:1_25:0) + CH_3COO	C_{45} H_{88} O5 N_1	22.77	Sphingolipids	1.19	< 0.05	13.34 ± 5.00a	4.77 ± 1.12c	7.66 ± 0.81b
Hex2Cer(d42:1) + CH_3COO	C_{56} H_{106} O_{15} N_1	19.63	Sphingolipids	1.15	< 0.05	8.95 ± 3.92a	3.95 ± 0.57c	6.48 ± 1.51b
PC(15:0_16:0) + CH_3COO	C_{41} H_{81} O_{10} N_1 P_1	14.52	Phospholipids	1.15	< 0.05	3.81 ± 1.11b	2.10 ± 0.34c	5.57 ± 2.08a
PC(16:0_18:3) + CH_3COO	C_{44} H_{81} O_{10} N_1 P_1	13.33	Phospholipids	1.46	< 0.05	3.34 ± 0.93a	2.66 ± 0.53b	1.84 ± 0.54c
PC(16:0_18:2) + CH_3COO	C_{44} H_{83} O_{10} N_1 P_1	14.42	Phospholipids	1.08	< 0.05	160.08 ± 28.23a	95.13 ± 22.51c	115.70 ± 14.07b
PE(18:1_12:0)-H	C_{35} H_{67} O_8 N_1 P_1	12.85	Phospholipids	1.83	< 0.05	0.92 ± 0.04c	1.70 ± 0.57b	2.26 ± 0.60a
PE(18:0_20:3)-H	C_{43} H_{79} O_8 N_1 P_1	16.50	Phospholipids	1.74	< 0.05	2.92 ± 1.08c	6.69 ± 2.32b	10.33 ± 4.76a
PI(18:1_18:1)-H	**C_{45} H_{82} O_{13} N_0 P_1**	**13.29**	**Phospholipids**	**1.64**	**< 0.05**	**13.12 ± 5.96c**	**21.43 ± 3.28b**	**28.35 ± 7.89a**
SM(d43:1) + CH_3COO	C_{50} H_{100} O_8 N_2 P_1	21.05	Sphingolipids	1.81	< 0.05	24.23 ± 11.42a	15.77 ± 2.60b	7.44 ± 7.08c
SM(t41:1) + CH_3COO	C_{48} H_{96} O_9 N_2 P_1	19.07	Sphingolipids	1.13	< 0.05	1.14 ± 0.91b	2.16 ± 0.67a	0.47 ± 0.29c
TG(19:1_18:1_18:2) + NH_4	C_{58} H_{108} O_6 N_1	24.34	Neutral lipids	1.74	< 0.05	3.99 ± 1.40a	2.72 ± 1.02b	1.73 ± 0.53c
TG(19:1_18:1_18:3) + NH_4	C_{58} H_{106} O_6 N_1	24.18	Neutral lipids	1.48	< 0.05	2.31 ± 1.01a	1.64 ± 0.64b	0.96 ± 0.48c
TG(18:1_12:0_18:3) + H	C_{51} H_{91} O_6	23.98	Neutral lipids	1.50	< 0.05	8.54 ± 2.62a	6.60 ± 1.32b	4.41 ± 1.29c
TG(6:0_10:0_17:1) + NH_4	**C_{36} H_{70} O_6 N_1**	**17.58**	**Neutral lipids**	**1.64**	**< 0.05**	**0.78 ± 0.36c**	**1.79 ± 0.14b**	**3.43 ± 1.66a**
TG(4:0_16:1_18:3) + NH_4	C_{41} H_{74} O_6 N_1	16.99	Neutral lipids	2.09	< 0.05	2.28 ± 1.26c	5.31 ± 2.87b	7.42 ± 2.14a
TG(11:0_18:1_18:2) + NH_4	**C_{50} H_{94} O_6 N_1**	**23.53**	**Neutral lipids**	**1.37**	**< 0.05**	**39.82 ± 10.63a**	**32.64 ± 5.24b**	**25.23 ± 4.00c**
TG(10:0_18:2_20:5) + H	**C_{51} H_{85} O_6**	**22.59**	**Neutral lipids**	**1.59**	**< 0.05**	**21.57 ± 4.95b**	**27.18 ± 4.29a**	**13.27 ± 2.81c**
TG(10:0_18:2_18:3) + NH_4	C_{49} H_{88} O_6 N_1	21.38	Neutral lipids	1.84	< 0.05	2.32 ± 1.71b	3.47 ± 0.89a	0.33 ± 0.31c
TG(18:1_13:0_18:2) + NH_4	C_{52} H_{98} O_6 N_1	23.90	Neutral lipids	1.54	< 0.05	40.16 ± 13.19a	27.42 ± 7.22b	19.18 ± 2.68c
TG(15:0_18:2_18:2) + NH_4	C_{54} H_{100} O_6 N_1	23.93	Neutral lipids	1.62	< 0.05	23.93 ± 9.97a	15.74 ± 5.25b	8.79 ± 2.43c
TG(4:0_8:0_12:0) + NH_4	C_{27} H_{54} O_6 N_1	11.18	Neutral lipids	1.90	< 0.05	66.51 ± 24.59c	105.77 ± 34.08b	143.86 ± 47.87a
TG(16:0_18:1_18:1) + NH_4	C_{55} H_{106} O_6 N_1	24.39	Neutral lipids	1.73	< 0.05	518.09 ± 156.19a	356.61 ± 108.65b	240.10 ± 51.34c
TG(18:1_12:0_18:1) + NH_4	C_{51} H_{98} O_6 N_1	24.01	Neutral lipids	1.84	< 0.05	612.96 ± 164.85a	397.80 ± 80.57b	296.45 ± 62.89c
TG(10:0_18:2_18:2) + NH_4	C_{49} H_{90} O_6 N_1	22.59	Neutral lipids	1.32	< 0.05	229.46 ± 72.60b	275.52 ± 36.46a	167.23 ± 29.59c
TG(18:1_14:0_18:2) + NH_4	C_{53} H_{100} O_6 N_1	24.04	Neutral lipids	2.15	< 0.05	315.11 ± 76.89a	193.35 ± 52.57b	132.38 ± 28.94c
TG(16:0_18:1_18:2) + NH_4	C_{55} H_{104} O_6 N_1	24.25	Neutral lipids	2.23	< 0.05	326.82 ± 78.89a	231.91 ± 68.08b	138.53 ± 35.90c
TG(16:0_14:0_18:3) + NH_4	C_{51} H_{96} O_6 N_1	23.75	Neutral lipids	1.76	< 0.05	249.39 ± 60.93a	191.74 ± 40.76b	133.32 ± 31.98c
TG(16:1_18:1_18:2) + NH_4	**C_{55} H_{102} O_6 N_1**	**24.07**	**Neutral lipids**	**1.87**	**< 0.05**	**196.39 ± 58.55a**	**134.62 ± 32.14b**	**72.73 ± 24.10c**
TG(14:0_18:2_18:2) + NH_4	C_{53} H_{98} O_6 N_1	23.78	Neutral lipids	2.36	< 0.05	107.03 ± 31.03a	76。80 ± 34.89b	40.83 ± 8.75c
TG(18:1_18:2_18:2) + NH_4	C_{57} H_{104} O_6 N_1	24.07	Neutral lipids	2.05	< 0.05	114.55 ± 47.42a	78.37 ± 33.71b	25.93 ± 23.94c
TG(16:1_18:2_18:2) + NH_4	C_{55} H_{100} O_6 N_1	23.83	Neutral lipids	1.46	< 0.05	35.11 ± 19.23a	21.66 ± 6.16b	9.54 ± 7.02c
TG(16:0_12:0_18:1) + NH_4	C_{49} H_{96} O_6 N_1	23.98	Neutral lipids	1.55	< 0.05	1050.77 ± 243.34a	803.41 ± 120.43b	647.99 ± 100.66c
TG(10:0_18:1_18:1) + NH_4	C_{49} H_{94} O_6 N_1	23.71	Neutral lipids	1.95	< 0.05	1107.60 ± 199.45a	963.12 ± 145.70b	659.97 ± 101.36c
TG(8:0_18:1_18:1) + NH_4	C_{47} H_{90} O_6 N_1	23.26	Neutral lipids	2.03	< 0.05	1833.36 ± 246.10c	1938.92 ± 265.13a	1143.33 ± 215.56b
TG(16:1_10:0_10:0) + NH_4	**C_{39} H_{76} O_6 N_1**	**18.84**	**Neutral lipids**	**1.29**	**< 0.05**	**953.88 ± 224.77c**	**1845.58 ± 256.41a**	**1530.93 ± 205.94b**
TG(10:0_14:0_18:1) + NH_4	C_{45} H_{88} O_6 N_1	23.19	Neutral lipids	1.96	< < 0.05	3227.44 ± 368.87a	2693.93 ± 382.17b	2288.30 ± 321.08c
TG(4:0_10:0_18:1) + NH_4	C_{35} H_{68} O_6 N_1	16.00	Neutral lipids	1.03	< 0.05	1232.56 ± 301.35c	2228.92 ± 442.61a	1871.04 ± 243.48b

The lipids in bold font were introduced in the established discriminate model.

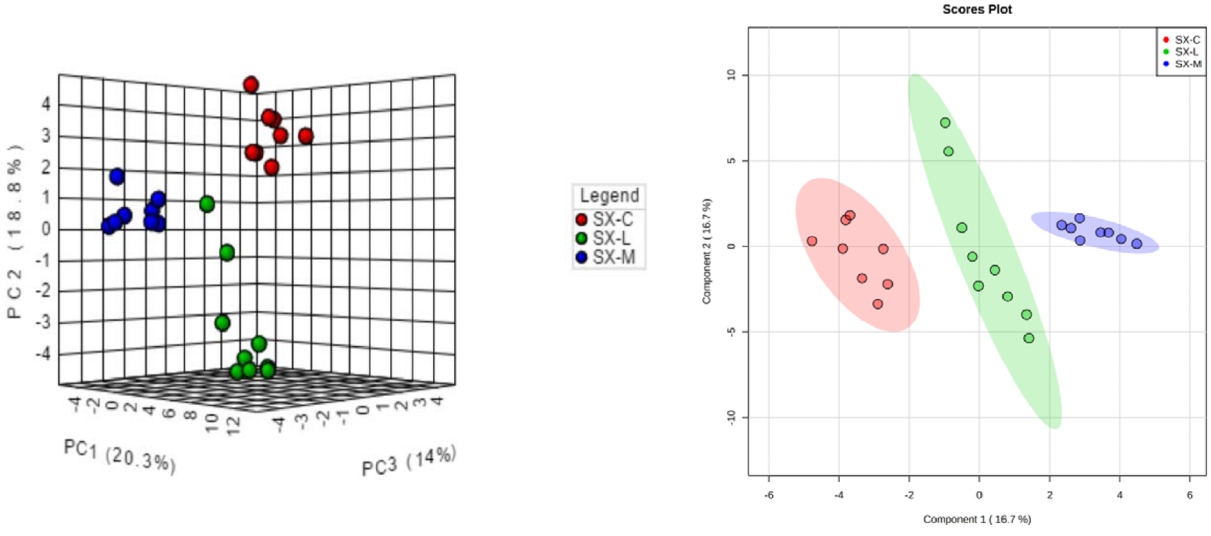

Fig. 3. PCA (A) and PLS-DA (B) of the lipidomic data obtained from colostrum, mature and late stage of goat milk samples.

Table 3
The potential lipid makers for discrimination of goat milk lactation stage.

Lipid	Formula	Retention time (min)	Lipid Category	VIP score	P value	Colostrum ($\times 10^6$)	Mature milk ($\times 10^6$)	Late milk ($\times 10^6$)
Cer(d18:1_22:1) + CH_3COO	C_{42} H_{80} O_5 N_1	19.16	Sphingolipids	2.67	< 0.05	5.05 ± 1.36 a	0.96 ± 0.15c	2.66 ± 1.12b
Cer(d18:1_18:1) + CH_3COO	C_{38} H_{72} O_5 N_1	16.10	Sphingolipids	1.19	< 0.05	3.24 ± 2.17b	4.91 ± 0.82 a	1.30 ± 1.16c
Cer(d16:1_24:1) + CH_3COO	C_{42} H_{80} O_5 N_1	18.92	Sphingolipids	2.92	< 0.05	3.31 ± 0.38a	0.57 ± 0.14c	1.91 ± 1.06b
Hex1Cer(d18:1_25:0) + CH_3COO	C_{51} H_{98} O_{10} N_1	21.52	Sphingolipids	1.08	< 0.05	7.21 ± 2.18b	10.43 ± 1.82a	4.77 ± 2.67c
Hex1Cer(d40:0) + CH_3COO	**C_{48} H_{94} O_{10} N_1**	**19.78**	**Sphingolipids**	**1.86**	**< 0.05**	**0.70 ± 0.21c**	**3.00 ± 1.38a**	**1.86 ± 1.13b**
PI(18:0_18:2)-H	C_{45} H_{82} O_{13} N_0 P_1	13.49	Phospholipids	2.47	< 0.05	8.28 ± 3.09c	29.66 ± 4.08a	21.05 ± 11.12b
SM(d37:1) + CH_3COO	**C_{44} H_{88} O_8 N_2 P_1**	**16.47**	**Sphingolipids**	**1.97**	**< 0.05**	**21.69 ± 5.57a**	**8.32 ± 1.46b**	**4.51 ± 2.79c**
SM(d35:1) + CH_3COO	C_{42} H_{84} O_8 N_2 P_1	14.59	Sphingolipids	1.46	< 0.05	35.24 ± 8.16a	16.44 ± 3.30b	8.78 ± 5.10c
SM(d36:1) + CH_3COO	C_{43} H_{86} O_8 N_2 P_1	15.68	Sphingolipids	1.07	< 0.05	198.25 ± 51.49a	134.75 ± 21.66b	65.73 ± 24.05c
TG(4:0_10:0_20:4) + NH_4	C_{37} H_{66} O_6 N_1	14.28	Neutral lipids	1.56	< 0.05	3.40 ± 1.00a	1.86 ± 0.88b	1.01 ± 0.39c
TG(6:0_6:0_20:4) + NH_4	C_{35} H_{62} O_6 N_1	12.90	Neutral lipids	1.28	< 0.05	4.56 ± 1.12a	2.80 ± 1.07b	1.57 ± 0.83c
TG(4:0_6:0_20:3) + H	**C_{33} H_{57} O_6**	**14.71**	**Neutral lipids**	**1.53**	**< 0.05**	**6.58 ± 2.46b**	**13.73 ± 3.23a**	**2.88 ± 1.74c**
TG(4:0_10:0_18:2) + NH_4	C_{35} H_{66} O_6 N_1	15.11	Neutral lipids	2.36	< 0.05	3.24 ± 1.99c	21.01 ± 6.24a	10.07 ± 3.47b
TG(4:0_6:0_16:0) + NH_4	**C_{29} H_{58} O_6 N_1**	**13.37**	**Neutral lipids**	**1.00**	**< 0.05**	**13.11 ± 7.90b**	**23.06 ± 8.57a**	**4.10 ± 3.47c**
TG(10:0_18:2_20:5) + H	C_{51} H_{85} O_6	22.59	Neutral lipids	1.76	< 0.05	17.75 ± 4.47c	27.18 ± 4.31a	22.54 ± 4.38b
TG(6:0_10:0_20:5) + H	**C_{39} H_{65} O_6**	**16.23**	**Neutral lipids**	**2.82**	**< 0.05**	**25.66 ± 8.52c**	**71.88 ± 6.79a**	**37.22 ± 8.71b**
TG(8:0_10:0_18:3) + NH_4	C_{39} H_{72} O_6 N_1	16.38	Neutral lipids	2.20	< 0.05	16.65 ± 8.01c	53.94 ± 14.98a	28.83 ± 9.50b
TG(4:0_18:0_18:1) + NH_4	C_{43} H_{84} O_6 N_1	22.54	Neutral lipids	1.24	< 0.05	205.12 ± 84.34b	377.62 ± 101.47a	101.74 ± 68.22c
TG(4:0_18:1_18:2) + NH_4	C_{43} H_{80} O_6 N_1	19.43	Neutral lipids	1.48	< 0.05	730.00 ± 247.37b	1128.11 ± 101.42a	418.00 ± 185.97c

The lipids in bold font were introduced in the established discriminate model.

100%. Fisher's discrimination functions for goat milk samples from different geographical origins are provided as follows:

$$Y_{Yunnan} = -8.484\ E-8\,PI20 + 3.61\ E-7\,TG30 + 6.470\ E-7\,TG179 - 7.629\ E-7\,TG184 + 1.560\ E-7\,TG334 + 1.684\ E-8\,TG394 - 28.684, \quad (1)$$

$$Y_{Shaanxi} = 1.047\ E-6\,PI20 + 2.274\ E-6\,TG30 - 1.057\ E-6\ TG179 + 2.342\ E-6\,TG184 - 4.173\ E-8\,TG334 + 3.725\ E-8\,TG394 - 60.500, \quad (2)$$

$$Y_{Shandong} = 1.639\ E-6\ PI20 + 6.771\ E-6\,TG30 - 5.335\ E-7\,TG179 + 5.442\ E-7\,TG184 - 1.447\ E-8\,TG334 + 2.895\ E-8\,TG394 - 54.467, \quad (3)$$

where PI20 is PI(18:1_18:1)-H; TG30 is TG(6:0_10:0_17:1) + NH_4; TG179 is TG(11:0_18:1_18:2) + NH_4; TG184 is TG(10:0_18:2_20:5) + H; TG334 is TG(16:1_18:1_18:2) + NH_4; TG394 is TG(16:1_10:0_10:0) + NH_4.

In order to discriminate goat milk samples of different lactation stages, two functions were selected and could explain 100% of the variance. Function 1 was mainly obtained by Hex1Cer(d40:0) + CH_3COO, TG(4:0_6:0_20:3) + H, TG(4:0_6:0_16:0) + NH_4 and TG(6:0_10:0_20:5) + H, and Function 2 was mainly obtained by SM (d37:1) + CH_3COO. The overall correct classification rate was 100%, whereas the cross-validation rate was 96%. Fisher's discrimination functions for goat milk samples of different lactation stages are provided as follows:

$$Y_{colostrum} = -6.370\ E-6\,H1C14 + 1.275\ E-6\,SM22 + 4.838\ E-6\,TG102 - 1.368\ E-6\,TG149 + 5.793\ E-7\,TG210 - 27.081, \quad (4)$$

$$Y_{Mature} = -1.981\ E-5\,H1C14 - 1.728\ E-6\,SM22 + 1.481\ E-5\,TG102 - 4.018\ E-6\,TG149 + 2.904\ E-6\,TG210 - 123.865, \quad (5)$$

$$Y_{Late} = -5.993\ E-6\,H1C14 - -6.121\ E-7\,SM22 + 4.323\ E-6\,TG102 - 1.280\ E-6\,TG149 + 1.152\ E-6\,TG210 - 19.206, \quad (6)$$

where H1C14 is Hex1Cer(d40:0) + CH_3COO; SM22 is SM(d37:1) + CH_3COO; TG102 is TG(4:0_6:0_20:3) + H; TG149 is TG(4:0_6:0_16:0) + NH_4; TG210 is TG(6:0_10:0_20:5) + H.

3.5. Application of the discrimination models

The Y value in each model is calculated by the abundances of lipid compounds and the coefficient in the model. When the abundances of the lipid compounds of one unknown goat milk were determined, the Y value in geographical origin discrimination model (formula (1)–(3)) or lactation stage discrimination model (formula (4)–(6)) can be deduced. The region of lactation stage corresponding to the maximum Y value is the geographical origin or lactation period to which the unknown goat milk belongs.

4. Discussion

In previous studies, lipidomics analysis was performed to identify the lipid composition in goat milk (Li et al., 2017) and the found lipid subclasses were roughly the same to those found in this study. In this study, additional lipid classes in goat milk were identified such as CL, HexCer and AcCa. The extraction method and determination method by Li et al. (2017) were adopted in the study. The differences might be ascribed to the difference in sample sources and the accumulated data of lipids. In this study, the significant differences in Cer and PC were found among the samples from different regions ($P < 0.05$) and extremely significant differences in PI, SM and FA were found among the samples from different regions ($P < 0.01$). Among the samples of different lactation stages, PG and TG were significant different and TG was extremely significant different.

The lipid differences among milk samples from different geographical origins could be associated with the dietary composition environment and feeding management (Capuano, Boerrigter-Eenling, Elgersma, & Ruth, 2014; Mele et al., 2016; Murphy, Mcneill, Connolly, & Gleeson, 1990; Thomson, Humphries, Kliem, Dittmann, & Reynolds, 2017; Yang et al., 2013). Milk fat contents as well as some individual fatty acids in cow milk were found to be significant different among different regions of China (Yang et al., 2013). Thomson et al. (2017) showed that the proportion of n-3 and n-6 polyunsaturated fatty acids in milk from dairy cows fed with a higher proportion of alfalfa silage were higher than those in milk from dairy cows fed with corn silage. Murphy et al. (1990) showed that full-fat soybeans increased the triglyceride content of C52 and C54 in milk. In addition, different feeds (fresh forage, forage silage, corn silage and concentrate) would lead different fatty acid patterns in milk TG and it was found that higher ratios of fresh forage could increase TG that containing 40, 52 and 54 acyl carbon atoms, and reduce the TG containing 34 and 36 acyl carbon atoms in milk (Capuano et al., 2014). Therefore, the abundances of TG (18:1_13:0_18:2) and TG (15:0_18:2_18:2) in goat milk from Yunnan and Shaanxi in our study were higher since the goats in these two farms were fed with more fresh pasture and soybeans.

In this study, the total fatty acid content of goat milk gradually increased during the lactation period. The similar result was also reported previously (Rako, Kalit, Kalit, Soldo, & Ljubenkov, 2018). In addition, the total saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids in goat milk also increased during the lactation period (Rako et al., 2018) and saturated fatty acids in cow milk and polyunsaturated fatty acids in donkey milk also showed the same trends (Martini, Altomonte, Manica, & Salari, 2015; Nantapo, Muchenje, & Hugo, 2014). Previous studies indicated that gangliosides composed of ceramide had the highest content in colostrum, the lower content in transition milk and mature milk, and the higher content in late milk (Martín, Martín-Sosa, & Hueso, 2001). Similarly, the Cer abundance in mature goat milk was found to be the lowest among three lactation stages in this study.

Based on PLS-DA and one-way ANOVA, the potential markers for identifying goat milk samples of different lactation stages from geographical origins were screened. Furthermore, the stepwise linear discriminant analysis was performed to establish discriminant models, six and five lipid molecules were respectively obtained for identifying the geographical origins and lactation stages and the discriminant effect was satisfactory. The result proved that lipidomics analysis was feasible and effective for discriminating goat milk in China.

5. Conclusion

The lipid classes and molecules in goat milk samples of different lactation stages from different geographical origins were determined and compared. Cer, PC, PI, SM were significantly different among goat milk samples from different geographical origins. PG and TG were significantly different among goat milk samples of different lactation stages. In total, 38 and 19 lipid molecules could be used as potential biomarkers to identify geographical origins and lactation stages of goat milk, respectively. Good discrimination results were obtained with the established models. The study provided the technical basis for milk authentication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125765.

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