Food Chemistry 245 (2018) 731–737

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Yak milk fat globules from the Qinghai-Tibetan Plateau: Membrane lipid composition and morphological properties

Jie Luo[a], Ziyu Huang[b], Hongna Liu[c], Yan Zhang[b], Fazheng Ren[a,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

[b] Key Laboratory of Functional Dairy, Co-constructed by Ministry of Education and Beijing Government, and Beijing Laboratory of Food Quality and Safety, China Agricultural University, Beijing 100083, China

[c] College of Life Science and Engineering, Northwest University for Nationalities, Lanzhou 730070, China

ARTICLE INFO

Keywords:
Yak milk
Milk fat globule membrane
Lipid domain
Phospholipid
Sphingomyelin
Cholesterol

ABSTRACT

Yak milk fat products constitute the base of Qinghai-Tibetan pastoralists' daily food intake. Despite the great importance of fat in processing and pastoralists' health, studies about yak milk fat are scarce. In this study, the lipid composition and the morphological properties of milk fat globule membranes (MFGMs) of yak milk were investigated. The results demonstrated that the yak milk had a higher cholesterol and sphingomyelin content compared to cow milk. In situ structural investigations performed at 25 °C by confocal microscopy showed the presence of lipid domains in yak MFGM, with a larger number and wider size range compared to cow milk. Moreover, the simultaneous localization of glycosylated molecules and polar lipids indicated that glycosylated molecules could be integrated into the lipid domains in yak MFGM. Different characteristics in yak MFGM could be related to the lipid composition and may affect the functions of yak milk lipids during processing and digestion.

1. Introduction

Yak is a bovid species that mainly lives in the Qinghai-Tibetan plateau at heights of 2500–6000 m above sea level. For centuries, yak milk is widely used to produce butter and ghee products, which constitute the base of Qinghai-Tibetan pastoralists' daily food intake and have been a major nutrient source for nomads. However, despite the great importance of fat to the properties of yak milk products and pastoralists' health, studies about yak milk fat are scarce.

Regardless of the milk origins, milk fat is considered to be dispersed in milk in the form of spherical droplets or globules, with a diameter naturally varying from < 0.2 to 15 μm. Compared with cow milk, yak milk contains fat in the range of 5.3–8.8% (w/v), which is almost twice that of cow milk (Li et al., 2011). Moreover, the average size of yak milk fat globules is significantly larger than cow milk (4.39 vs. 3.87 μm) (Luo, Wang, Song, Pang, & Ren, 2016). Previous studies have showed that milk contains higher fat content and larger fat globules that are more predisposed to coalescence during processing (Wiking, Björck, & Nielsen, 2003). Therefore, it is much easier for the yak milk fat to coalescence and creaming during processing, making it a perfect source for making butter and ghee products.

In addition to the size and content differences, the physical stability of fat globules towards coalescence is largely dependent on the properties of the milk fat globule membrane (MFGM). MFGM is formed during fat secretion when the fat droplets are enveloped by a three-layered phospholipids membrane structure. The MFGM acts as a natural emulsifying agent, which is highly structured and contains unique polar lipids (Phosphatidylcholine, PC; Phosphatidylethanolamine, PE; Sphingomyelin, SM; Phosphatidylinositol, PI and Phosphatidylserine, PS), membrane-specific proteins (mainly glycoproteins), triglycerides, sterols (mainly cholesterol) and glycolipids (Lopez, 2011). The use of confocal laser scanning microscopy (CLSM) (Evers et al., 2008) and subsequently together with the exogenous fluorescent phospholipid Rd-DOPE (Gallier, Gragson, Jiménez-Flores, & Everett, 2010; Lopez, Madec, & Jimenez-Flores, 2010) offer the possibility to measure the phase separation of polar lipids in MFGM. Non-fluorescent domains observed in the MFGM were believed to be the lateral segregation of polar lipids in ordered phases (either the rigid liquid-ordered (Lo) or the gel phase) (Et-Thakafy, Guyomarc'h, & Lopez, 2017; Nguyen et al., 2015; Zheng, Jiménez-Flores, Gragson, & Everett, 2014). The Lo phase involves high Tm saturated polar lipids (e.g., SM, saturated *PC* and saturated PE) and cholesterol, while the gel phase only contains the high Tm polar lipids (Et-Thakafy et al., 2017). The fluorescent part of the MFGM corresponds to the fluid matrix mostly composed of

* Corresponding author.
E-mail addresses: lizzy-154@163.com (J. Luo), huangziyu@cau.edu.cn (Z. Huang), lhnxbmz@126.com (H. Liu), zhangyan282@sina.com (Y. Zhang), renfazheng@263.net (F. Ren).

https://doi.org/10.1016/j.foodchem.2017.12.001
Received 18 August 2017; Received in revised form 29 October 2017; Accepted 2 December 2017
Available online 05 December 2017
0308-8146/

glycerophospholipids (PC, PE, PI, PS) with unsaturated fatty acids and a very low Tm, called the liquid-disordered (Ld) phase (Lopez et al., 2011). The preponderance of saturated hydrocarbon chains allows the molecules to be tightly packed in the Lo phase and the gel phase (Lopez et al., 2010), which has been shown to have higher resistance to rupture compared to the Ld phase (Murthy, Guyomarc'h, & Lopez, 2016a). This fragility may correspond to the breakdown point of the MFGM during milk processing (e.g., during the churning of cream) (Lopez et al., 2011), which is extremely important for yak milk for its application in butter products. Furthermore, polar lipids such as SM have been shown to have important biological functions such as gut maturation and myelination of the newborn's developing central nervous system (Spitsberg, 2005). However, the phospholipid content and its individual constitution in yak milk has not yet been revealed, while whether the lipid domains exist in the yak MFGM and their molecular arrangement on it also remain to be elucidated.

The objective of this work was to investigate the lipid composition and the membrane morphological characteristics in situ in yak and cow milks. CLSM was used to investigate the microstructure with adapted fluorescent dyes able to simultaneous localization the polar lipids, glycoproteins and glycolipids located within the MFGM. By comparing yak milk with cow milk, the relationship between the lipid composition of the MFGM and its microstructure was also revealed.

2. Materials and methods

2.1. Milk samples

Bulk tank raw yak milk was obtained from the Tianzhu grassland on the Qinghai-Tibetan Plateau in June (mid-lactation of yak). Bulk tank raw cow milk was obtained from SanYuan Dairy Co. (Beijing, China) at the same time. After milking, 0.02% (w/v) sodium azide was immediately added into the milks and kept at 4 °C. All milk samples were used to perform the analysis within 2 days. The fat content in the milk samples was determined according to Marshall's method (Marshall, 1992).

2.2. Measurement of ζ-potential of fat globules

The ζ-potential of freshly collected samples was measured by laser Doppler velocimetry and phase analysis light scattering (M3-PALS) technique using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Malvern, UK). Samples were diluted 100-fold in 20 mM imidazole, 50 mM of NaCl, 5 mM of $CaCl_2$, and a pH 7 buffer and put in a special cuvette at 20 °C. Ten readings from a freshly diluted individual sample were collected and the measurements were run in triplicate on three independent milk samples.

2.3. Measurement of particle size distribution

A Mastersizer 3000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Malvern, UK) was used to determine the average droplet size in milk samples. Milk samples were restored up to room temperature (20 °C) before measurements. The casein micelles were dissociated by adding 1 mL of 35 mM EDTA/NaOH and pH 7 buffer to the milks, in the apparatus. The refractive indices used were 1.333 for water, while the refractive indexes of fat globules were 1.460 at 466 nm and 1.458 at 633 nm. A pinch of samples was added to 500 mL distilled water in the measurement cell of the apparatus in order to reach 10–20% obscuration. From the size distribution, the average volume-weighted diameter:

$$D_{4,3} = \sum nidi^4 / \sum nidi^3$$

and the weighted average surface diameter:

$$D_{3,2} = nidi^3 / \sum nidi^2$$

were determined where *ni* is the number of fat globules in a size class of diameter *di*.

The specific surface area of milk fat globules (m^2/g fat), were calculated by the instrument software.

2.4. Extraction and analysis of the lipids

2.4.1. Chemicals and reagents

For high-performance liquid chromatography (HPLC) and gas chromatography (GC), all the chemicals and reagents were chromatographic grade. The phospholipid standards were supplied by Sigma-Aldrich: PE (L-α-phosphatidylethanolamine dipalmitoyl, N,N-dimethyl (C16:0), purity 99%), PI (L-α-phosphatidylinositol ammonium salt from soybean, purity 98%), PS (1,2-diacyl-*sn-glycero*-3 phosphoserine, purity 98%), PC (1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine, purity 99%) and sphingomyelin (SM from bovine brain, purity 99%). Cholesterol standard and low melting point agarose were also supplied by Sigma-Aldrich.

2.4.2. Extraction of total lipids

Milk samples were centrifuged at 3000 *g* for 5 min at 4 °C, and the top cream layer was kept. Two grams of cream was mixed with 40 mL of chloroform/methanol (2:1, v/v) and submitted to continuous slow agitation at 4 °C for 1 h using a magnetic stirring apparatus. The extract was shaken with 10 mL of 0.73% NaCl (w/w) and equilibrated in a separating funnel. The upper phase was washed three times with a mixture of 40 mL of chloroform/methanol (2:1, v/v) and 10 mL of NaCl 0.58% (w/w). The lower solvent phases were kept and filtered (Whatman filter paper, 2.5 µm, Grosseron, France) and evaporated under vacuum. Total lipids extracted were stored at −20 °C until further analysis.

2.4.3. Analysis of polar lipids

The analysis of polar lipids were performed according to the adjusted method (Lopez et al., 2011). The total lipid extraction sample (100 mg) was dissolved in 500 µL of chloroform/methanol (2:1, v/v) using silica gel-bonded columns (Supelco LC-Si 0.5 g, Supelco Bellefonte, USA). The determination of the polar lipid classes was then performed using an Agilent 1260 Infinity HPLC system equipped with a Nucleosil 50–5 column (250 × 3 mm; 5 µm) and a precolumn in silica (8 × 3 mm; 5 µm) was used. The settings were a column temperature: 50 °C; gas flow rate: 1 L/min; evaporating temperature: 90 °C; injection volume: 50 µL; mobile phase A: ammonium formate 3 g/L; and mobile phase B: acetonitrile/methanol (100:3). The elution program was stated with A/B 1:99 (v/v) to 30:70 (v/v) from 0 min to 19 min, then equilibrated for 2 min, and back to the initial conditions of 1:99 over 3 min. The total chromatographic run time was 24 min. The flow rate was maintained at 1 mL/min. Each sample was injected three times. The identification of the polar lipids was carried out by a comparison with the retention times of the pure standards. The sum of glycerophospholipids and SM content was regarded as the total phospholipid content.

2.4.4. Analysis of cholesterol

Cholesterol was determined by GC (Shimadzu, Japan) according to the method described in Chinese Standard (2016). For preparation of cholesterol standards, the stock solution (1 mg/mL) was prepared by dissolving 50 mg of cholesterol standard with 50 mL ethanol. Working solutions were prepared by appropriately diluting aliquots from the stock solution with hexane to obtain solutions in the range of 0.01–0.5 mg/mL. GC injection parameters: 1 µL, splitless; 200 °C for 1 min; 30 °C/min to 280 °C, 280 °C isothermal for 10 min. DB-5 capillary column (30 m × 0.32 mm × 0.25 µm, Agilent, CA, USA); injection temperature, 280 °C; detector temperature, 290 °C.