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The interfacial properties of various milk fat globule membrane components using Langmuir isotherms

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ABSTRACT

In an attempt to understand the interfacial properties of various milk fat globule membrane (MFGM) components, their monolayers were characterized using a Langmuir film balance. MFGM fractions were separated into lipids (total and polar lipids) and defatted mass. The interfacial properties of these components were characterized at the air/water interface. The interfacial properties of MFGM were characterized at both the air/water and oil/water interfaces. Using the compression isotherm, the surface elasticity of MFGM components was also calculated. It was found that MFGM polar lipids showed relatively high surface elasticity which confirms their potential to help stabilize food systems.

1. Introduction

Lipids in milk are present in the form of globules dispersed in the aqueous phase. Milk fat globule membranes (MFGM) cover the fat globule to ensure its structural integrity. Quantitatively the MFGM accounts for 2–6% of these fat globules (Singh, 2006). MFGM has a tripartite structure, with proteins and polar lipids forming the major component of the primary monolayer and secondary bilayer (Vanderghem et al., 2011). Previous studies have suggested that proteins account for 25–60% of MFGM. The lipids isolated from MFGM contain 56–80% neutral lipids and 15–43% polar lipids (Vanderghem et al., 2010).

Many researchers have reported on the health promoting and techno-functional properties of the MFGM. Some researchers suggested that the MFGM can be used as a functional food ingredient due to its emulsifying and stabilizing properties (Corredig & Dalgleish, 1997, 1998a, 1998b; Dzul-Cauich, Lobato-Calleros, Pérez-Orozco, Alvarez-Ramirez, & Vernon-Carter, 2013; Phan et al., 2013; Phan, Le, Van der Meeren, & Dewettinck, 2014). These functional properties of MFGM are probably due to the presence of proteins and polar lipids.

The Langmuir film balance is an efficient tool for characterizing the monolayers and intermolecular interactions (Bos & Nylander, 1996; Fernández, Sánchez, Rodríguez Niño, & Rodríguez Patino, 2007; Li et al., 1998; Polverini et al., 2003). Work has been done to study the surface properties of the MFGM at the air/water interface using the Langmuir film balance (Danthine & Blecker, 2014; Innocente, Blecker, Deroanne, & Paquot, 1997). Gallier, Gragson, Jiménez-Flores, and Everett (2010) used polar lipids from dairy sources (not directly extracted from MFGM) to mimic MFGM components and studied their monolayers at the air/water interface. However, no report was found that specifically focused on analyzing the interfacial properties of individual MFGM components.

In this study, the surface properties of different MFGM constituents (obtained from MFGM fractions isolated at different stages) were characterized using a Langmuir film balance at the air/water interface. These compression isotherms were used to calculate the surface elasticity. This surface elasticity was correlated with the emulsion and foam stabilizing properties of different MFGM components and fractions. The properties of MFGM monolayers were also studied at the oil/water interface. The objective of this study was to determine the surface properties of MFGM components using a langmuir film balance, to gain understanding of their mechanism in stabilizing some food systems. Moreover, the chemical composition and physical properties of MFGM fractions obtained at different stages of isolation are being reported for the first time through the outcomes of this study.

2. Materials and methods

2.1. Raw material

Fresh cream (bovine milk) was purchased from a local farm having 20 cows from three breeds (Montbéliarde, Normade and Holstein). The milk was obtained using automatic milking systems and was centrifuged at 2500*g* (3191M, Alfa Laval, Lund, Sweden) to obtain cream.

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Average fat content of cream was 44 ± 2%. A low melting anhydrous fat fraction (iodine value 42, melting temperature 15 °C) was provided by Corman SA (Goé, Limbourg, Belgium). Detailed characterizations of this fraction were previously published by Danthine (2012).

2.2. Extraction of MFGM fractions

MFGM fractions were isolated according to the method described by Vanderghem et al. (2008) with slight modifications. Proteins (casein and whey proteins) and lactose in the cream were removed by washing twice with saline phosphate buffer (0.01 M Na_2HPO_4/NaH_2PO_4; 0.9% NaCl; pH 7.2 (pH meter 320/ Set 2, WTW, Weilheim, Germany)). Residual phosphate buffer in the cream was washed away using distilled water. Between these washes, the cream was centrifuged (Avanti J-E, Beckman Coulter Inc., Indianapolis, IN, USA) at 4500*g* (Rotor JA-14, Beckman Coulter Inc.) for 10 min at 4 °C. The washed cream was then re-suspended in distilled water to obtain the same weight as the original raw cream. The cream was then aged (20 h at 4 °C). Then, 1.25 kg of cream was churned using a mixer (KM300, Kenwood Ltd., Havant Hants, Britain) fitted with a 5 wire stainless steel whisk in a stainless steel bowl of 4.4 L capacity at speed 4, for about 15 min until the MFGM suspension, i.e., serum phase (F1) and fat phase were separated. The fat phase was washed with warm distilled water (45 °C) to recover the residual serum phase (F2). Both F1 and F2 were warmed (30 min at 45 °C) and centrifuged (5000*g* for 15 min at 4 °C) to remove the residual fat. F1 and F2 were freeze dried (Model DW 8, Heto-Holten A/S Gydevang, AllerØd, Denmark) and stored at −50 °C until further analysis for up to 30 wk. These fractions were mixed in the same ratio as recovered to obtain a reconstituted MFGM fraction (F3). To ensure that the sample was representative, the MFGM were isolated from fresh cream in ten batches of 2.5 kg (during 4 months spanning from February to May 2015).

2.3. Compositional analysis and separation of MFGM components

The nitrogen content of F1, F2 and F3 was analyzed using a Dumas method (Rapid N cube, Elementar Analysensysteme GmbH, Hanau, Germany) and multiplied by 6.38 to get the protein content (International Organisation for Standardization, 2002). This is the general Kjeldahl factor for dairy products and may or may not be appropriate for MFGM. Proteins were characterized using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Amersham Biosciences, Little Chalfont, United Kingdom). SDS-PAGE was done using an EPS 3500 XL - Electrophoresis Power Supply Unit (Pharmacia LKB, Uppsala, Sweden). Solutions were loaded in 12.5% polyacrylamide gels (GE17-0623-01, GE Healthcare, Buckinghamshire, UK) and runs were terminated when the running front reached the end of the gel. Bromophenol blue (Sigma-Aldrich NV, Bornem, Belgium) dye was used to identify the dye front. Visualization of gels was done using Coomassie Brilliant Blue G 250 (Sigma-Aldrich NV) staining. A low molecular weight SDS marker kit (LMW-SDS marker kit, GE Healthcare) was used to identify different proteins.

Total lipids from F1, F2 and F3 were extracted using a 2:1 chloroform:methanol solution (v/v) (Rombaut, Camp, & Dewettinck, 2005). The lipids obtained from F1, F2 and F3 were designated as F1 Lipid, F2 Lipid and F3 Lipid, respectively. Similarly, the defatted residues (after lipid extraction) obtained from F1, F2 and F3 were designated as F1 Defat, F2 Defat and F3 Defat, respectively.

Quantitative fractionation of the total lipids into different classes was done using an aminopropyl column (HF Mega BE-NH2, 2 g, 12 mL, Agilent Technologies, Santa Clara, CA, USA) (Vaghela & Kilara, 1995). A vacuum pump (Millivac™ Mini Vacuum Pump, Millipore Corp., Billerica, MA, USA) was used to elute solvents from column under 10 kPa vacuum. The column was loaded with 70 mg lipids dissolved in 2 mL chloroform. Neutral lipids, free fatty acids and polar lipids were eluted using 18 mL each of 2:1 chloroform:2-propanol (v/v), 2% (v/v) acetic acid in diethyl ether and methanol, respectively. The neutral lipid fraction obtained was dissolved in 1 mL hexane and loaded on a second column. Cholesterol esters, triglycerides, cholesterol, diglycerides and monoglycerides were eluted using 12 mL hexane, 36 mL 1% (v/v) diethyl ether and 10% (v/v) methylene chloride in hexane, 36 mL 5% (v/v) ethyl acetate in hexane, 36 mL 15% (v/v) ethyl acetate in hexane and 18 mL 2:1 (v/v) chloroform:methanol, chloroform: methanol, respectively. The polar lipid fractions obtained from F1, F2 and F3 were designated as F1 PL, F2 PL and F3 PL, respectively.

The analysis of polar lipid classes in F1 PL, F2 PL and F3 PL was done using a HPLC system (20A series, Shimadzu, Tokyo, Japan) connected to an evaporative light scanning detector (ELSD model 3300, Alltech Associates Inc., Lokeren, Belgium) (Le et al., 2011). All the polar lipids standards were purchased from Sigma-Aldrich NV. The mobile phase consisted of solvent A (dichloromethane) and solvent B (acetic acid: trimethylamine buffer (pH 4.5, 500:21 (v/v))) in the gradient. The gradient used was as follows: 0 min (96% A, 4% B), 4 min (88% A, 12% B), 12 min (6% A, 94% B) and 17–22.5 min (96% A, 4% B). The flow rate of the mobile phase was 0.5 mL/min, the column oven temperature was 40 °C and the sample chamber temperature was 20 °C. The injection volume was 10 μL. The concentration of polar lipids in samples were determined by using known concentrations of polar lipids standards (Sigma-Aldrich NV) to construct a linear standard curve for each polar lipid.

Lactose was analyzed using the dinitrosalicylic acid reagent method (Miller, 1959). Lactose in the sample was incubated at 100 °C for 10 min after addition of 100 μL 1.5 N HCl. The reaction was terminated by addition of 100 μL 1.5 M NaOH and 30 μL distilled water. The sample was then incubated at 100 °C for 5 min following the addition of 3 mL of dinitrosalicylic acid (Alfa Aesar, Karlsruhe, Germany). This reaction was terminated by addition of 1 mL of 40% sodium potassium tartrate. After cooling, 15 mL of distilled water was added to the samples and absorbance was measured at 550 nm (UV-2401 PC, Shimadzu, Kyoto, Japan). Concentration of lactose was determined by using a known concentration of lactose previously hydrolyzed to construct a linear standard curve.

2.4. Interfacial studies

Compression isotherms were obtained using a Langmuir film balance (Liquid-liquid Langmuir trough, KSV NIMA Instruments Ltd., Espoo, Finland). Compression isotherms allow determination of the surface pressure as a function of decreasing surface area with compression which reflects the packing of the monolayer at the interface. When a monolayer is spread, it exists in the gaseous phase, i.e., molecules in the monolayer do not interact with each other. On compression, the molecules come closer and an increase in surface pressure is observed (A0). At this point, the monolayer still is in the gaseous phase. The surface pressure increases until an inflection point denoted as the liquid expanded phase (A1, $\pi 1$) occurs. Upon further compression, the monolayer undergoes a transition to a liquid condensed phase and then a solid phase at which a second transition phase is observed (A2, $\pi 2$). On further compression of the monolayer; a horizontal break in the isotherm is observed which can be considered as the maximum surface pressure at which the monolayer collapses (At, πt). These different physical states of monolayers are related to the confirmation of molecules and intermolecular interactions (Eeman & Deleu, 2010).

MilliQ water (18.2 mΩ cm) ((MilliQ water; Millipore Corp., Bedford, MA, USA) was used as the sub phase (the liquid filled in the Langmuir trough) in all the analyses. MFGM fractions and defatted residues were dissolved in MilliQ water (4 mg/mL), and stored at 4 °C overnight; while the polar lipid and lipid fractions were dissolved in chloroform (2 mg/mL) for the analysis.

Air/water interface: The solutions of polar lipids (10 μL), complete lipids (10 μL), defatted residues (80 μL) and MFGM fractions (60 μL) were spread at the air/water interface using a micro syringe. The