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Impact of technological processes on buffalo and bovine milk fat crystallization behavior and milk fat globule membrane phospholipids profile

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ABSTRACT

The effects of milk technological processes (pasteurization, homogenization, and freeze-drying) on the crystallization and melting behaviors of buffalo and bovine milk fat were investigated. As well, the profile of milk phospholipids was identified. Differential scanning calorimetry was used to evaluate the variations in the thermal behaviors of milk fat. Milk phospholipids were purified by the solid-phase extraction method, and then identified by using an ultra-performance liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry. Seven classes of phospholipids (including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, sphingomyelin, and lysophosphatidylcholine) were identified. Milk processing affected both the thermal characteristics and the profile of phospholipids, since the content of phosphatidylcholine increased after the homogenization of pasteurized milk. The finding of this study could provide a valuable knowledge to dairy industry and the related applications since it highlights the variations in milk fat crystallization and melting behaviors, and the profile of phospholipids throughout a series of technological treatments.

1. Introduction

Among the different mammals, buffaloes and cows are the main dairying species, and their milk is conventionally the most produced kind of milk (Fox, 2002). Buffalo milk is a plentiful source of nutrients, comprising higher concentrations of almost all the major components than bovine milk (Huppertz, Upadhyay, Kelly, & Tamime, 2006). Buffalo milk represents approximately 10% of the worldwide milk production, while bovine milk represents about 84% (IDF, 2010). Milk fat is considered one of the major milk components, which regulates the particular properties of high-fat content dairy products. It has a functional role in bakery and confectionery industries. The different applications require specific characteristics of milk fat, which in turn needs improved functionality control (Herrera, de Leon Gatti, & Hartel, 1999). The type and amount of milk-fat crystals at the temperature of application have a significant effect on milk fat functional properties. Milk fat is characterized by the existence of approximately more than 400 different molecular species of triacylglycerols with fatty acids comprising 2–24 carbon atoms (Gresti, Bugaut, Maniongui, & Bezard, 1993). The structure of milk triacylglycerols is responsible for the crystallization behavior, melting points, and rheological properties of milk fat as globules (Kaylegian & Lindsay, 1995).

Due to the large number of triglycerides with a wide range of chain lengths and degrees of saturation, milk fat demonstrates a wide-ranging melting points varying in temperature from −40 to 40 °C, and usually showing three overlapping endotherms. Milk fat mainly contains high-melting fractions, middle-melting fractions, and low-melting fractions. Knowledge of the chemical composition, phase behavior, and polymorphism of these fractions and their mixtures and how their properties influence each other would be helpful to understand, predict, and control the physical properties of milk fat (Marangoni & Lencki, 1998). The phase behavior of milk fat seemed to be complicated because of the polymorphism of the solid phase (Ten Grotenhuis, Van Aken, Van Malssen, & Schenk, 1999). Previous studies on milk fat thermal behaviors by using differential scanning calorimetry (DSC) indicated that specific groups of triacylglycerol molecules strongly affect the melting

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and crystallization characteristics (Lopez, Bourgaux, Lesieur, Riaublanc, & Ollivon, 2006). DSC provides a rapid, sensitive, and reproducible fingerprint technique for the characterization of edible oils and fats (Tan & Man, 2002).

Phospholipids (PLs) are an important group of biomolecules. They are predominantly located in the milk fat globule membranes (MFGM), and in the membrane compounds of the skim milk. PLs play an essential role in milk through the emulsification of fat in the aqueous phase owing to their amphiphilic characteristics (Contarini & Povolo, 2013). The content of PLs in milk and dairy products not only affected by the properties of the raw material, but the applied technological processes as well. Because PLs mainly existed in MFGM, their composition and distribution in the final product would be affected by any treatment that causes a perturbation of the membrane and/or a separation or fractionation of fat globules, such as homogenization and/or centrifugation of polar and neutral components of fat. The variations in milk components during heat treatments (Ye, Singh, Taylor, & Anema, 2002, 2004), and homogenization (Cano-Ruiz & Richter, 1997; Zamora, Ferragut, Guamis, & Trujillo, 2012) have been reported. Different analytical techniques have been widely applied for the separation and the identification of milk PLs, for example solid-phase extraction and high-performance liquid chromatography with evaporative light scattering detector (Avalli & Contarini, 2005), hydrophilic interaction liquid chromatography coupled to evaporative light scattering and mass spectrometry detection (Donato et al., 2011), ^{31}P nuclear magnetic resonance (Giuffrida et al., 2013), liquid chromatography-matrix assisted desorption ionization mass spectrometry (Walczak, Pomastowski, Bocian, & Buszewski, 2016), and ultra-performance liquid chromatography- quadrupole-Exactive Orbitrap mass spectrometry (Li et al., 2017). Ultra-performance liquid chromatography with quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS) has been recently used in complicated systems analysis owing to its rapid and sensitive separation, and the precision in mass measurement. The current advances and modifications in this method of analysis resulted in better separations with faster analysis, high resolution, and increased sensitivity; consequently, complex lipids separation and characterization problems could be solved (Laaksonen et al., 2006).

Milk and dairy products undergo a series of technological treatments for specific purposes, such as killing the pathogenic micro-organisms, reducing the size of fat globules, and increasing the shelf-life of the final products, etc. The technological and organoleptic properties of dairy products are correlated with the responses of MFGM to milk processing (Ye et al., 2002). Many publications have reported the composition of PLs in raw milk but, in general, there is a lack of data on PLs content of processed dairy products. This study aimed to investigate the influences of different technological processes (pasteurization, homogenization, and freeze-drying) on the crystallization and melting profiles of milk fat, and the molecular species composition of PLs from buffalo and bovine milk.

2. Materials and methods

2.1. Materials

Raw milk samples from Egyptian buffaloes (*Bubalus bubalis*) and Egyptian cattle (*Bos aegyptiacus*) were collected from local farms (Zagazig, Sharkia, Egypt). PLs standards were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Silica gel cartridges (CNWBOND Si, 3-mL volume, 500 mg sorbents) were purchased from CNW Technologies Inc. (China). All chemicals and solvents used in extraction and analysis were of high purity and of analytical grade. Methanol (LC-MS grade), acetonitrile (LC-MS grade), and chloroform (HPLC grade) were provided by TEDIA (Tedia Company Inc. Ohio, USA).

2.2. Milk technological processes

Raw milk; one fourth of buffalo and bovine milk was separated for analysis as raw buffalo milk (RBM) and raw bovine milk (RCM). Pasteurized milk; the remaining milk was kept in double walled vessels at 63 ± 0.5 °C in a heating water bath maintained at 65 °C. After 30 min of heating the samples were cooled directly to 4 °C. The resultant milk was analyzed as pasteurized buffalo milk (PBM) and pasteurized bovine milk (PCM). Homogenized milk; after pasteurization, milk was homogenized in a two-stage homogenizer (ATS Engineering Inc. Nano homogenizer AH-2010). The homogenization pressure was set at 150 and 100 bars in the first stage and the second stage, respectively. The resultant milk was analyzed as homogenized buffalo milk (HBM) and homogenized bovine milk (HCM). Freeze-dried milk; pasteurized homogenized milk was finally freeze-dried in a laboratory-scale freeze-dryer (Xianou-12 N freeze-dryer, Nanjing Xianou, Ltd.), the temperature was set at −83 °C and the pressure was adjusted at 1 Pa. The resultant milk was analyzed as freeze-dried buffalo milk (FBM) and freeze-dried bovine milk (FCM).

2.3. Total lipids extraction and phospholipids purification

The total lipids were extracted from milk samples following the procedure reported by Folch, Lees, and Sloane-Stanley (1957). Samples were dissolved in 200 mL chloroform:methanol (2:1, v/v) mixture. The mixture was then mixed for 15 min and centrifuged at 3500 rpm for 15 min. The organic phase containing milk lipids was collected and equilibrated with ¼ volume of a saline solution (NaCl 0.86%, w/w). The lower chloroform layer was filtered and evaporated under reduced pressure, and the obtained total lipids were stored at −20 °C until further analysis. PLs fractions form different treatments were purified by using the solid-phase extraction method reported by Avalli and Contarini (2005). After the purification, 5–10 mg of milk PLs was re-dissolved in 1 mL chloroform:methanol (2:1, v/v) for UPLC-Q-TOF-MS analysis.

2.4. Crystallization and melting profiles

Differential scanning calorimetry (DSC Q2000 V4.7A Build 121, TA Instruments, New Castle, DE, USA) was used to analyze the crystallization and melting profiles of milk fat. During the analysis, nitrogen gas was used to purge the system at 20 mL/min, at the same time nitrogen was used to serve as a refrigerant for cooling the system. The calibration was carried out by using indium, eicosane, and dodecane standards. 5–10 mg of milk fat sample was hermetically sealed in an aluminum pans, and an empty pan was used as a reference. The applied time-temperature program for the non-isothermal crystallization was as follows: holding at 60 °C for 15 min to ensure a totally liquid state, cooling at 10 °C/min to −60 °C for raw milk and −40 °C for processed milk, holding for 5 min, and then heating at 20 °C/ min to 60 °C.

2.5. Ultra-performance liquid chromatography-mass spectrometry

The analysis of milk PLs was carried on ultra-performance liquid chromatography (UPLC) system (Waters, Milford, Massachusetts, USA) equipped with an hydrophilic interaction liquid chromatography column (2.1 × 100 mm, 1.7 μm particle size). Acetonitrile was used as mobile phase A, and 10 mM ammonium acetate as mobile phase B. Efficient separation of PLs was achieved with a binary gradient starting with 80% A: 20% B for 8 min, then changing to 60% A: 40% B and holding for 10 min, and finally 100% A for 10 min; the flow rate was 0.3 mL/min. The temperature of the column and sample room was set at 45 °C.

A quadrupole time-of-flight (Q-TOF) mass spectrometry (MS) instrument (Waters, Milford, Massachusetts, USA) was used to identify and quantify PLs fractions in the different samples. Negative-ion (-ve)